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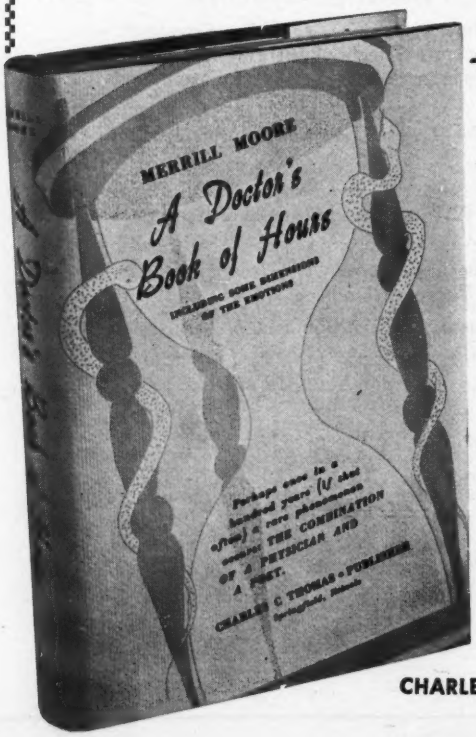

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CHROMOSOMAL SEX IN GONADAL DYSGENESIS (OVARIAN AGENESIS): RELATIONSHIP TO MALE PSEUDOHERMAPHRODISM AND THEORIES OF HUMAN SEX DIFFERENTIATION*†

MELVIN M. GRUMBACH, M.D.,‡§ JUDSON J. VAN WYK, M.D.‡¶ AND LAWSON WILKINS, M.D.

The Department of Pediatrics, Johns Hopkins University School of Medicine, and the Harriet Lane Home, The Johns Hopkins Hospital, Baltimore, Maryland

CONSIDERABLE knowledge has been accumulated concerning the factors affecting sex differentiation in vertebrates. In man, however, most of our information is inferential, based largely on the observations of experimental embryologists in other species. Controversy and debate have focused principally on the relative role of genetic influences and the embryonic gonads in the differentiation of the genital ducts and the external genitalia. The demonstration by Jost in the rabbit (1), and Raynaud and Frilley in the mouse (2), that early removal or destruction of the fetal gonads led invariably to female development of the ducts and genitalia, emphasized the importance of the embryonic testes in counteracting the

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‡ Fellow of the National Foundation for Infantile Paralysis.

§ Present address: Babies Hospital, Columbia-Presbyterian Medical Center, New York, N. Y.

¶ Present address: North Carolina Memorial Hospital, Chapel Hill, N. C.

inherent tendency of the fetus to feminize. Individuals with the syndrome of "ovarian agenesis" had always been considered to be females. In 1950 Wilkins (3) and Jost (4), recognizing the clinical significance of these animal experiments, predicted that some patients with this disorder should be chromosomal males.

Barr and his associates have observed a difference in the nuclear chromatin pattern of somatic cells in the male in contrast with the females of certain species of mammals (5–8). Examination of human epidermis has provided a practical technique for the determination of chromosomal sex in man (9, 10). The application of this method to the study of patients with the syndrome of "ovarian agenesis," reported in preliminary communications by the authors (11) and others (12) indicated that some patients with so-called ovarian agenesis are chromosomal males.

The present study concerns 22 patients with this syndrome, correlating the sex chromatin pattern with the clinical findings and with concepts of human sex differentiation and related problems. In addition, 2 atypical cases are presented which serve to indicate and elucidate a relationship between chromosomal males with what has been called previously the syndrome of "ovarian agenesis" and classic forms of male pseudohermaphrodism.

Numerous designations have been applied to this syndrome and are currently used, such as ovarian agenesis (13), Turner's syndrome (14), Bonnevie-Ullrich syndrome (15, 16), primary ovarian insufficiency with decreased stature (17), and the syndrome of rudimentary ovaries (18). In view of the observation that patients with this disorder may be either chromosomal males or females, titles implying only an ovarian defect or deficiency should be abandoned. Moreover, since rudimentary mesonephric elements of the gonad are present in the genital ridge, in actuality there is dysgenesis rather than agenesis of the gonads. Therefore, we advocate the title *gonadal dysgenesis* and, to distinguish the variant of this syndrome in which some degree of intrauterine masculinization of the external genitalia has occurred, *gonadal dysgenesis with phallic enlargement.*[1] This nomenclature will be employed in the remainder of this paper.

SUBJECTS, METHODS AND RESULTS

Table 1 shows the essential clinical and laboratory findings in 22 patients with the syndrome of gonadal dysgenesis studied in this clinic. These pa-

[1] Gordon and his associates introduced the term gonadal dysgenesis to describe 2 patients with "ovarian agenesis" who had "clitoral" enlargement (19). However, for the reasons stated, we feel the term gonadal dysgenesis should be applied generically to the entire group of patients with this disorder, and the few with an enlarged phallus appropriately designated as a subgroup.

tients had normal, but infantile female external genitalia and there was no evidence of female secondary sexual characteristics. Representative data have been selected from these patients, many of whom have been followed in this clinic over a period of years. Included are the results of skin biopsy for chromosomal sex in each, and the findings on exploratory laparotomy in 5 subjects; 2 of the latter have been reported previously by Wilkins and Fleischmann (13) and the typical pathologic findings described in detail. The authors have recorded the major congenital malformations[2] detected on physical examination and those which were revealed by special roentgenographic studies, performed when indicated.

Biopsy specimens of skin were prepared according to the technique of Moore, Graham and Barr (9), and both Feulgen and hematoxylin-and-eosin stains of sections were made. The validity of this method had been confirmed by other workers (20, 21). All sections were reviewed by, and studied in conjunction with Dr. Murray Barr and his coworkers at the University of Western Ontario. The urinary 17-ketosteroids were determined by a modification of the method of Callow, Callow and Emmens (22); gonadotropins (FSH) were assayed by the method of Bradbury, Brown and Brown (23), based on the technique of Klinefelter *et al.* (24).

In Table 2 certain data are summarized in two age groups—patients under 6 years of age, and from 10 to 30 years of age.

Patients from 10 to 30 years of age: In this group of 17 patients with normal female external genitalia and absence of estrogenic manifestations, the diagnosis of gonadal dysgenesis was confirmed by finding an elevated gonadotropin titer in all 16 of the patients on whom this determination was performed, and by exploratory laparotomy in 5 instances. Fifteen of these individuals had a male chromatin pattern, and 2 a female pattern.

Patients under 6 years of age: The diagnosis in the 5 apparently female patients in this group, aged 2 months to 5½ years, was not verified by laparotomy or by gonadotropin assay. In 4, the peculiar habitus, localized edema of the distal portions of the extremities such as described by Ullrich (16), and the cutis laxa or webbing of the nuchal region suggested the diagnosis. The fifth child was studied merely because of striking dwarfism without osseous retardation in an apparent female. All 5 individuals were chromosomal males.

Coarctation of the aorta was observed in 5 of the 22 patients in this study. As might be predicted from the rarity of this malformation as an isolated defect in the female (25), these subjects were all chromosomal

[2] Worthy of note was the finding of multiple telangiectasia of the small intestine at exploration in 2 cases and unexplained hypertension in 2 cases. Exhaustive studies in 1 of the latter, including retrograde and intravenous pyelography, renal arteriograms, and a benzodioxane test were all unrevealing.

TABLE 1. SALIENT FINDINGS IN 22 CASES OF TYPICAL GONADAL DYSGENESIS

Patient	Age (yrs.)	Height (in.) / Height age (yrs.)	Bone age (avg. yrs.)	Birth wt. (lbs.)	Sex. infantilism (female)	Pubic hair (scanty)	Habitus and malformations										FSH (m.u./day)	17-KS (mg./day)	Skin biopsy	
							Short stature	Mature facies	Micro-gnathia	Shield-like chest	Webbed neck	Coarctation of aorta	Edema*	Skeletal anomalies	Osteo-porosis	Misc.†			Male	Female
A.S.‡	17	53.3 / 10	14	"7"	+	+	+	+	0	+	+	0	0	0	+	0	>576	2.6	+	
B.Wg.‡	16	53.5 / 10	14	6.1	+	+	+	+	+	+	0	0	0	+	+	1, 18	> 96 <192	4.0	+	
M.L.‡	30	57.5 / 12	18+	8.5	+	+	+	±	+	0	±	0	0	0	+	2, 3, 4, 5	—	2.4	+	
M.H.‡	16	50.0 / 8 9/12	14.5	"5"	+	+	+	+	+	+	+	+	0	+	+	6, 7, 8	>85	2.2	+	
B.W.	15	58.0 / 12	15	11.1	+	+	+	+	+	+	+	+	+	+	+	6, 8, 9, 10, 11, 18	> 96 <192	8.1	+	
M.Le.‡	22	57.0 / 11 9/12	—	?	+	+	+	±	0	0	±	0	+	0	—	4.8	>210	5.6	+	
J.B.	17	56.7 / 11 6/12	16	?	+	+	+	+	+	+	0	0	0	0	+	0	>105.6	—	+	
J.N.	16	55.8 / 11 3/12	14	"4"	+	+	+	+	0	+	0	0	0	+	0	0	>105.6	1.6		+
C.T.	14 2/12	52.3 / 9 9/12	12 at 12 4/12	5.8	+	+	+	+	+	+	±	0	0	0	0	8	> 52.8	2.9	+	
S.F.	14 0/12	50.2 / 8 9/12	12	4.5	+	+	+	+	0	+	0	0	0	0	0	0	> 70 <178	1.2	+	
E.A.	14 0/12	51.2 / 9 3/12	14	5.2	+	+	+	+	+	+	0	0	0	+	+	12, 13	>288 <576	3.9	+	

M.H.	$13^{3}/_{12}$	$\frac{48.5}{7^{6}/_{12}}$	13	6.0	+	+	+	+	+	0	+	+	0	+	+	10	>288 <576	2.9	+	
E.K.	$13^{11}/_{12}$	$\frac{49.0}{8}$	12	4.8	+	+	+	+	0	0	0	0	0	±	0	0	>105.6	1.1		+
J.M.	$12^{6}/_{12}$	$\frac{50.8}{9}$	11	4.6	+	0	+	+	0	+	0	0	±	0	+	1, 14	>105.6	2.7	+	
G.F.	$11^{1}/_{12}$	$\frac{46.7}{7}$	8 at $8^{6}/_{12}$	6.3	+	+	+	+	0	+	0	0	0	0	0	8, 10, 15, 18	> 52.8	—	+	
B.L.	$10^{9}/_{12}$	$\frac{45.5}{7}$	10	6.8	+	0	+	+	+	+	+	0	+	0	0	5, 6	> 52.8 <105.6	1.9	+	
M.N.	$10^{1}/_{12}$	$\frac{48.5}{7^{9}/_{12}}$	10	7.5	+	0	+	+	+	+	+	+	+	0	0	5, 11, 18	> 52.8	0.6	+	
Patients under 6 years of age																				
J.E.	$5^{6}/_{12}$	$\frac{36.5}{3}$	4	7.1	+	0	+	+	+	+	+	0	+	+	0	5, 8, 11, 18	< 6.6	0.2	+	
C.L.	$3^{10}/_{12}$	$\frac{38.0}{3^{6}/_{12}}$	$3^{1}/_{2}$	6.1	+	0	0	+	+	+	+	0	+	+	0	10, 11	—	—	+	
L.S.	$1^{8}/_{12}$	$\frac{23.2}{2/_{12}}$	$1^{4}/_{12}$	2.8	+	0	+	+	0	0	0	0	0	0	0	0	—	—	+	
S.B.	$1^{6}/_{12}$	$\frac{31.0}{1^{5}/_{12}}$	$2^{6}/_{12}$	7.6	+	0	0	+	+	+	+	0	+	0	0	5, 9, 16, 18	—	—	+	
D.S.	$^{2}/_{12}$	$\frac{21.2}{1/_{12}}$	$^{4}/_{12}$	7.4	+	0	0	0	0	0	±	+	+	0	0	9, 17	—	—	+	

* Edema of the distal portions of the extremities (Ullrich 15, 16).

† The key to the figures in this column is as follows:

1—unexplained hypertension
2—auricular septal defect
3—deafness (?nerve)
4—multiple telangiectasia of small intestine
5—epicanthal folds
6—mental retardation
7—congenital ptosis
8—cubitus valgus
9—cutis laxa
10—microthelia
11—deformity of auricle and pinna
12—webbing of toes
13—café-au-lait spots and molluscum fibrosum
14—congenital squint
15—congenital cataracts
16—hypertrophic pyloric stenosis (infantile)
17—duplication of ureter
18—high arched palate

‡ Laparotomy revealed a vestigial gonad (genital ridge) with complete absence of germinal elements and scanty mesonephric elements; the Fallopian tubes, uterus and vagina were normally formed but infantile.

TABLE 2. FINDINGS IN GONADAL DYSGENESIS ACCORDING TO AGE

Age group	Under 6 years	From 10 to 30 years
No. of cases	5	17
Short stature	2	17
Normal or sl. delayed bone development	5	16/16
Webbed neck	3	6
Coarctation of aorta	1	4
Edema*	4	4
Elevated FSH	0/1	16/16
Laparotomy	0	5
Chromosomal sex		
Male	5	15
Female	0	2

* Involving distal portions of extremities (Ullrich (15)).

males. The 2 chromosomal females had the characteristic habitus, but few congenital anomalies[3]; neither had a webbed neck.

CASE REPORTS

The following 2 patients are described in detail because the anatomy of the genital tract or the external genitalia differed from that usually found in gonadal dysgenesis. The interpretation of these findings has elucidated the varying stages of transition between this syndrome and male pseudohermaphrodism.

Case 1. Gonadal dysgenesis with phallic enlargement

H.G. (H.L.H. #B 3122), a 13-year-old female, was referred to the Harriet Lane Home because of failure to develop any female secondary sexual characteristics, shortness of stature, and an abnormality of the external genitalia. The parents were of average height and there was a normal 7-year-old male sibling. No history of miscarriage, unusually short collaterals, sterility, or endocrine disorders was obtained. The pregnancy was full-term and uneventful and the patient weighed 4 pounds, 8 ounces at birth. No significant diseases occurred during childhood. Growth had proceeded at a regular but relatively slow rate. An enlarged clitoris was observed at birth but had not shown any tendency to increase in size. No signs of excessive androgenic secretion were observed during childhood. At 11 years of age pubic hair began to develop and shortly thereafter axillary hair, but during the next two years no signs of female pubescence ensued.

On physical examination she was an intelligent, short, stocky girl with mature facies,

[3] However, Barr (26) has studied a chromosomal female with this syndrome, who had a webbed neck and an obscure cardiac defect.

a broad chest, square shoulders, and narrow hips. Figure 1 shows the patient after eighteen months of estrogen therapy.

Anthropometric measurements were as follows:

Age: $13\frac{1}{12}$ yrs.	Patient	Average for height	Average for age
Height	56.4 in.	Height age $11\frac{1}{2}$ yrs.	59.7 in.
Weight	90.0 lbs.	72.2 lbs.	90.0 lbs.
Span	54.5 in.	56.3 in.	59.7 in.
Symphysis to sole	27.0 in.	28.2 in.	30.0 in.
Upper/lower segment	1.09	1.00	0.99
Circumference head	21.2 in.	20.9 in.	21.2 in.
Circumference chest	30.0 in.	26.6 in.	28.1 in.

The blood pressure was 90/60 in the right arm and the pulse rate 98 per minute Twenty-eight permanent teeth had erupted.

Sexual development: The areolae and nipples were infantile and widely separated on the broad chest. No breast tissue was palpable. A few dark axillary hairs and scanty amounts of coarse, dark, curly labial and pubic hair were present. The clitoris was enlarged and measured 2.5 cm. × 1 cm. It was covered by a large preputial fold. The labial majora were not excessively developed or wrinkled. The labia minora appeared infantile and joined the preputial folds. Separate urethral and vaginal orifices were present and visible within the vestibule. No gonads were palpable in the labia majora or the inguinal region. No acne, seborrhea, hirsutism, or excessive muscular development was observed. The voice was feminine.

The rest of the findings on physical examination were entirely within normal limits, except for a high arched palate.

Pertinent laboratory studies: The skeletal age was 13 years, utilizing the average of multiple centers of ossification (27). Urinary 17-ketosteroids were 4.6 and 4.8 mg. per twenty-four hours. The titer of gonadotropins (FSH) was greater than 26.4 and less than 52.8 mouse units per twenty-four hours—a high titer for this age, particularly in an individual without signs of puberal estrinization. A skin biopsy specimen obtained at a later date showed a male chromatin pattern in the epidermal nuclei.

The adrenogenital syndrome was excluded by the fact that the urinary 17-ketosteroids were not elevated and there was no evidence of excessive androgenic secretion and signs of progressive virilization. The enlarged clitoris combined with otherwise female external genitalia suggested a form of ambisexual development and an exploratory laparotomy was recommended.

Findings at operation: Dr. Howard Jones of the Department of Gynecology performed a cystoscopic examination and abdominal exploration. On cystoscopy a normal appearing urethra and bladder were found. Examination of the vagina with the endoscope showed identifiable but atrophic vaginal rugae. At the apex of the prepuberal sized vagina the cervix was visible. Figure 2 is a drawing of the pelvic organs found at operation. The uterus was small, measuring about 3.5 cm. in length and less than 1 cm. in breadth and could be palpated more easily than seen. Arising from both cornu and lying in a normal position in the broad ligament were normal sized preadolescent fimbriated Fallopian tubes. On the posterior surface of each broad ligament, in the location normally occupied by the ovaries, a long whitish ridge was seen which measured about

4 cm. in length and less than 3 mm. in diameter. This ridge was similar to the primitive genital ridges seen in other cases (13), except for being broader and more irregular in some areas, with spotty tan discoloration. Careful exploration showed that no true testes or ovaries were present. The kidneys and adrenals were of normal size and consistency.

A bilateral salpingectomy including removal of the primitive genital streak was performed, as it was apparent that the child would never be fertile. The clitoris was not sufficiently enlarged to warrant removal and since the genital ridges were removed, there seemed no probability that further increase in size would occur.

Microscopic examination: The histologic findings were similar bilaterally. Figure 3a is

FIG. 1. Case 1 (H.G.) at 14½ years of age, *after eighteen months of estrogen therapy.* The enlargement of the phallus is not readily seen in this photograph.

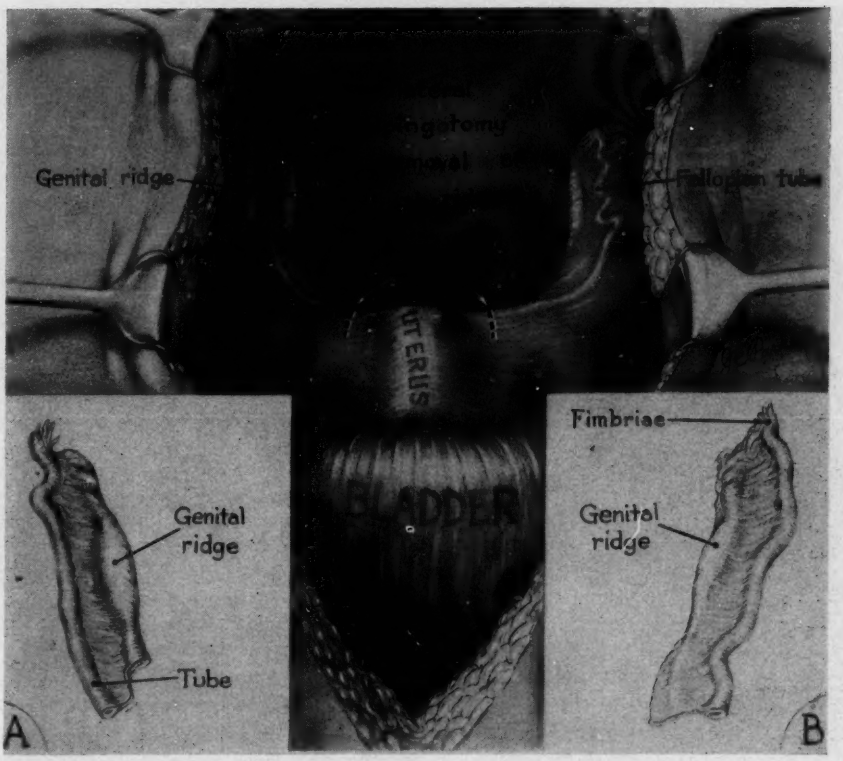

FIG. 2. The appearance of the pelvic structures at operation in Case 1 (H.G.), showing the relationship of the genital ridges to the infantile Fallopian tubes and uterus.

a low-power photomicrograph of a section of the left genital ridge and illustrates the absence of germinal elements beneath the ridge and the abundant mesonephric vestiges in the medullary portion and in the mesosalpinx. In Figure 3b the genital ridge is seen under high power and shows the spindle shaped connective-tissue stroma arranged in whorls, the complete absence of germinal elements, and the presence of nests of mature appearing epithelioid cells varying in shape, with intensely pink staining cytoplasm and eccentrically placed nuclei. The cells contained brown pigment but no lipoid droplets or Reinke's crystalloids (the latter usually are not seen before puberty) (Fig. 3c). These cells, which we believe are Leydig cells, were arranged in clusters or infiltrated throughout the hyaline stroma. In the deeper layers numerous tubules were found (Fig. 3d). Some tubules had a single layer of cuboidal cells, others were formed of columnar epithelium which occasionally invaginated into the lumen and had smooth muscle coats. These were probably rete tubules and other tubules derived from the mesonephros.

The Fallopian tubes were typical of those found during preadolescence. The epithelial lining was columnar and no ciliated cells were seen.

Course: After estrogen therapy for five months and then cyclical estrogen and

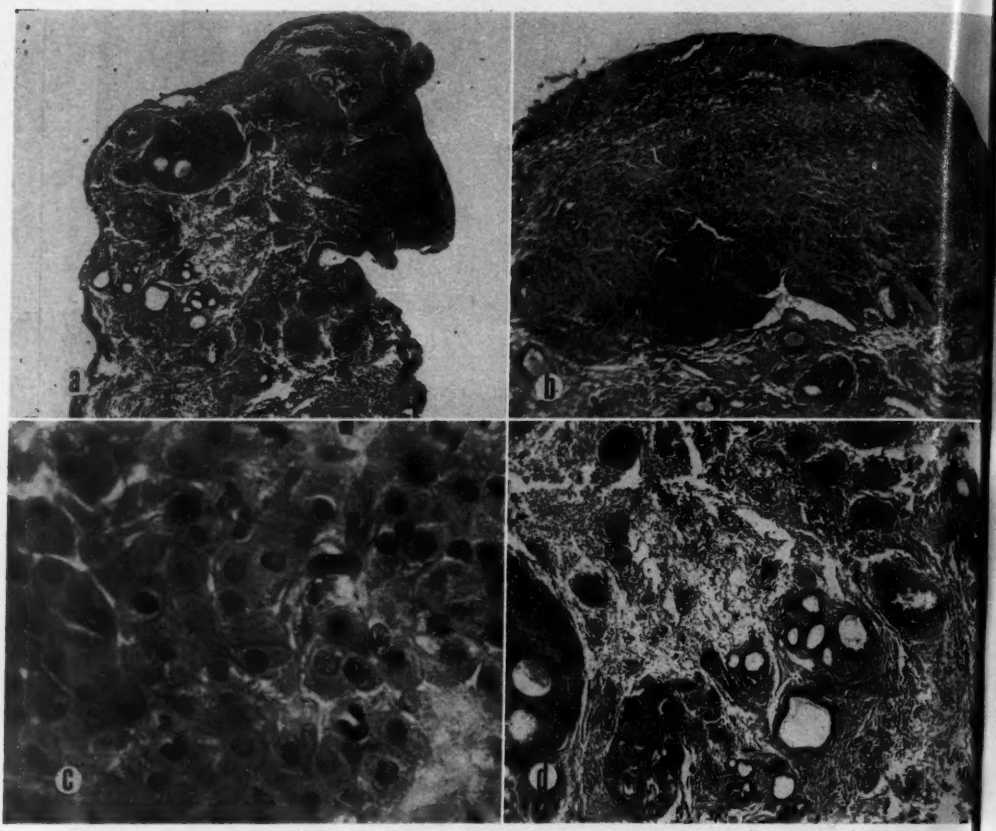

FIG. 3. Microscopic appearance of the genital ridge in Case 1 (H.G.) **a.** Low-power view of the genital ridge and a portion of the mesosalpinx (H & E; ×15). Note the abundant mesonephric elements, and the vestigial vas deferens, in the upper left-hand border of the section. **b.** Higher power photomicrograph (H & E; ×65) of genital ridge, illustrating the absence of germinal elements (primordial follicles or seminiferous tubules), the clusters of Leydig cells, and the wavy gonadal stroma. **c.** High-power view of the epithelioid (Leydig) cells in the medullary region (H & E; ×485). **d.** The appearance of the rete and other tubules in the medullary region (H & E; ×45).

progesterone treatment, the patient began to menstruate regularly. Breast development, estrinization of the vaginal smear, and development of the vagina and labia minora were satisfactory. A 1.3-inch gain in stature occurred over the next nine months. It is anticipated that the patient will be able to lead a normal sexual life as a female, except for fertility.

Comment: In retrospect, many of the initial findings were entirely compatible with the diagnosis of gonadal dysgenesis, such as (a) short stature, (b) normal skeletal age,

(c) mature features, (d) high arched palate, (e) absence of breasts or development of the labia minora, (f) presence of a small amount of pubic hair, (g) elevated gonadotropin titer in the urine, and (h) the urinary 17-ketosteroids of 4.5 mg. In addition the patient was a chromosomal male. The unusual finding with this syndrome was the presence of an enlarged clitoris or genital tubercle. We have called this variant of the syndrome, *gonadal dysgenesis with phallic enlargement.*

"Clitoral" enlargement previously has been described in patients with gonadal dysgenesis (18, 28), but it is a rare occurrence. Gordan and his associates recently have reviewed the literature and reported two additional cases (19). In the medullary region of the genital ridges, nests of Leydig cells are found, although some authors have considered these cells as of adrenocortical origin (18). Small clumps of Leydig cells frequently can be found in the genital ridges of cases without phallic enlargement, and occasionally these cells are present in relatively large numbers (29, 30). Whether or not enlargement of the phallus is present at birth would seem to be related to the functional activity of the Leydig cells in the embryonic testes.

Case 2. Male pseudohermaphrodite

G.C. (H.L.H. #B 15566), an $8\frac{5}{12}$-year-old girl was referred to the Harriet Lane Home because of doubt about her "true sex." All other members of the family, including the father, mother, an older sister and a younger brother were of average height, in good health and free of any physical abnormalities. No family history of dwarfism, sterility or endocrine disorders was obtained. The patient was born after a full-term pregnancy and weighed 5 pounds 8 ounces. She had been free of illness except for the usual childhood diseases. Although she had always been short for her age, the rate of growth had been steady and mental development normal.

The patient was born with a hypospadic phallus and raised as a boy for the first three years of life. At this time an exploratory laparotomy was performed at another hospital. Because of the presence of an "ovary and tube," the sex of the child was changed and she was subsequently reared as a girl. No biopsy of the gonad was made. The patient regarded herself as a girl. However, she had become concerned about her enlarged phallus and because of an uncertainty on the part of the parents as to whether the patient was a girl or a boy, she was referred for a definite opinion. No pubic hair had developed nor was there any evidence of progressive virilization or breast development.

Figure 4 is a photograph of the patient. She appeared as a child, short for her age, with relatively mature features, a prominent forehead, a short broad-based neck, and a shield-like chest with well separated nipples. Her measurements were as follows:

Age: $8\frac{5}{12}$ years	Patient	Average for height	Average for age
Height	45.0 in.	Height age 6 yrs.	49.9 in.
Weight	46 lbs.	42 lbs.	54.6 lbs.
Span	42.0 in.	43.3 in.	49.6 in.
Symphysis to sole	21.1 in.	20.0 in.	24.5 in.
Upper/lower segment	1.13	1.12	1.04
Circumference head	20.5 in.	20.1 in.	20.5
Circumference chest	23.0	22.0	23.8

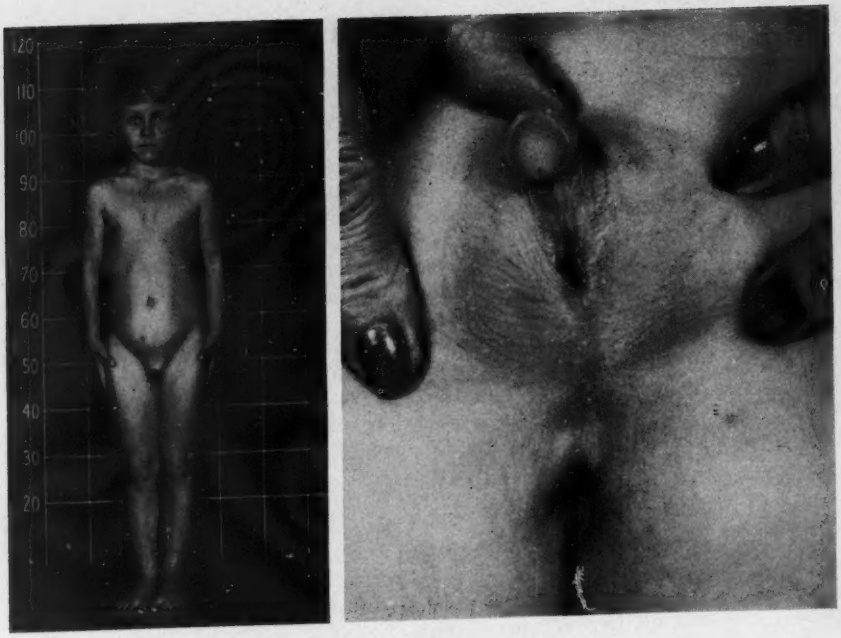

FIG. 4

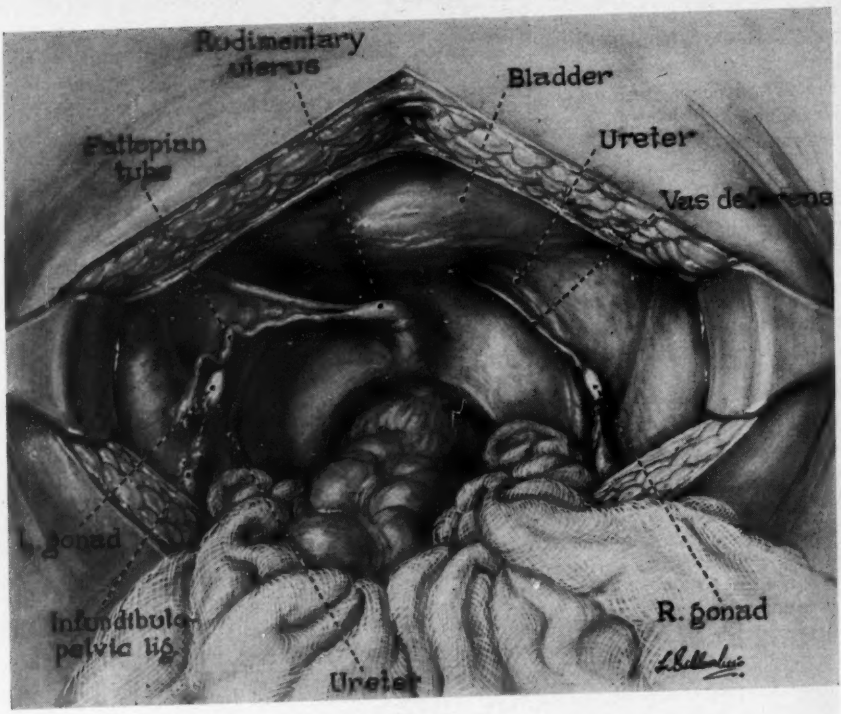
Rudimentary uterus
Bladder
Fallopian tube
Ureter
Vas deferens
L. gonad
Infundibulo-pelvic lig.
Ureter
R. gonad

FIG. 5

The blood pressure was 120/80 in the arms and 145/105 in the legs; the pulse was 80 per minute. The dental age was normal.

Sexual development: The nipples were infantile and no breast tissue was felt. No acne, seborrhea, excessive muscular development, hirsutism, axillary or pubic hair was observed.

The external genitalia presented an hermaphroditic appearance. There was a hypospadic phallus measuring 3.5 cm. in length and 1.5 cm. in diameter, bound in chordee with a redundant prepuce (Fig. 4). A small funnel-shaped, single orifice was visible at the base of the phallus. A thin fold of skin extending posteriorly and inferiorly from the glans formed the lateral margins of the orifice. The labia majora were fleshy, slightly wrinkled, fused in the midline, and had a distinctly scrotal appearance. No gonads were palpable in the labia majora or the inguinal region. On rectal examination no uterus or gonads were felt but a small, firm, flat midline mass about 0.5 cm. in diameter was present, which possibly was an infantile prostate.

The rest of the findings on physical examination were normal.

Pertinent laboratory studies: The skeletal age, using an average of multiple centers of ossification, was 7½ years (27). Urinary 17-ketosteroids ranged from 0.5 to 1.5 mg. per twenty-four hours. A specimen of skin from each arm showed a male chromatin pattern on microscopic examination of the epidermal cells.

To clarify the nature of the patient's ambisexual development and to initiate plastic correction of the external genitalia to conform to that of a female, laparotomy was performed. For psychologic reasons, no change in sex was planned (31, 32).

Findings at operation: Dr. Howard Jones performed the cystoscopy and exploratory laparotomy. Examination of the urethra with an endoscope passed through the hypospadic orifice showed a normal urethra and bladder. No opening into a vagina was demonstrable. A structure was visualized in the midline of the posterior urethra which was interpreted as the verumontanum. Figure 5 illustrates the findings on laparotomy. On the left side of the pelvis, in the position normally occupied by the ovary, a small smooth white oval-shaped mass measuring about 7 mm. in length by 4 mm. in width was found. In the midline, between the bladder and rectum and extending slightly to the left, a small smooth mass was seen which appeared to be a Müllerian structure, and on microscopic examination proved to be a rudimentary uterus. Arising from the left cornu of this structure was a hypoplastic, infantile, fimbriated Fallopian tube. When the rudimentary uterus was dissected, a tiny vestigial vagina was revealed. The latter disappeared between the bladder and rectum and its inferior attachment was not ascertained. The right portion of the midline Müllerian body was strikingly less well differentiated than the left. No structure resembling a Fallopian tube was identified on this side. However, more laterally on the right side of the pelvis where normally the ovary would be found, a narrow small smooth white structure about 6 mm. in length and 3 mm. in breadth was seen. Attached to this mass and passing medially and inferiorly toward the prostatic region there was a fine white duct about 1 mm. in diameter, which

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FIG. 4. Case 2 (G.C.), at $8\frac{5}{12}$ years of age. Note the broad-based neck, widely separated nipples, short stature, and the hypospadic phallus with cryptorchidism.

FIG. 5. The operative findings in Case 2 (G.C.) Note the infantile Fallopian tube on the left side, the rudimentary uterus, and the hypoplastic vas deferens extending inferiorly into the pelvis from the right gonad.

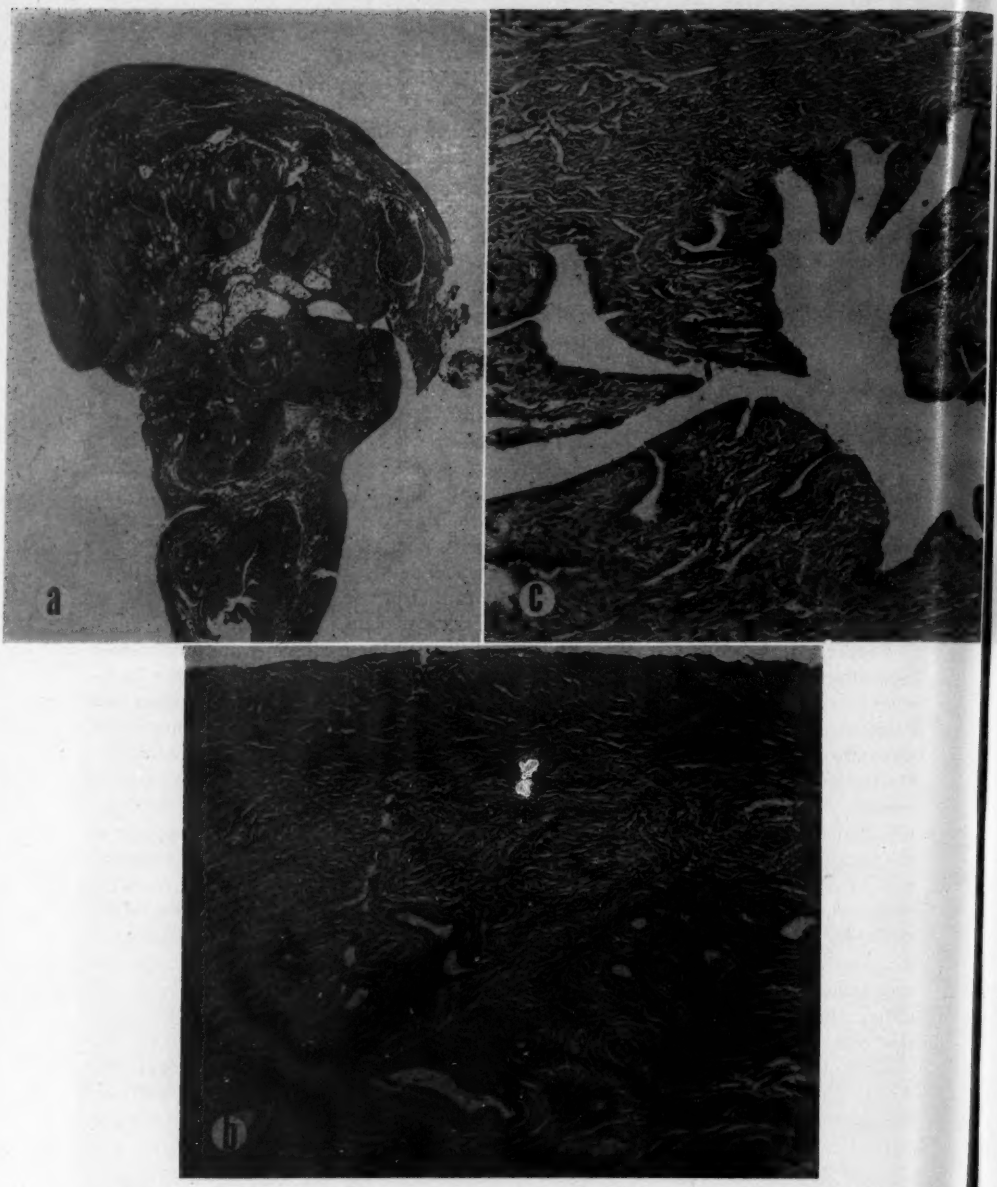

FIG. 6. The gonad and the genital duct on the left side in Case 2 (G.C.). **a.** Low-power photomicrograph of the left gonad and the Fallopian tube (bottom of photograph) (H & E; ×15), displaying the general relations and the abundant mesonephric elements in the gonad and the mesosalpinx. **b.** Higher power magnification of the left gonad (H & E; ×150), illustrating the absence of germinal elements, the wavy gonadal stroma, and the rete (mesonephric) tubules. **c.** Infantile left Fallopian tube (H & E; ×200).

proved to be a vas deferens. The gonads and genital-duct structures were removed for study and a clitorectomy performed. At a later age a vagina will be constructed.

Microscopic examination: The essential microscopic findings are shown in Figures 6 and 7. Figure 6a is a low-power view through the left gonad and the Fallopian tube illustrating the general relation of these structures. A high-power microphotograph of the left gonad (Fig. 6b) illustrates the absence of germinal elements and the wavy gonadal stroma. In the deeper layers, tubules without muscular coats and lined with a cuboidal or low columnar nonciliated epithelium were present, which resembled rete tubules. Although carefully searched for, no Leydig cells were seen. Deeper in the medulla and in the adjacent mesosalpinx, tubules of mesonephric origin similar to those shown in Case 1 were found. Figure 6c is a section through the left Fallopian tube and shows the prepuberal epithelial lining. The gross appearance of the right gonad was similar to that of the left. However, a high-power view (Fig. 7a) of the right gonad revealed a few scattered clusters of immature seminiferous tubules in the medullary region, surrounded by wavy gonadal stroma. These tubules (Fig. 7b) were small, without lumina, and were lined with one to two layers of undifferentiated cells. A few cells with clear cytoplasm were seen in the basal layers, which resembled spermatogonia. Figure 7c is a cross section of the right vas deferens.

Comment: This child presented some of the stigmata associated with the syndrome of gonadal dysgenesis, namely, a) short stature, b) normal bone age, c) shield-like chest, and d) broad-based neck. However, there was a hypospadic phallus, instead of the infantile female external genitalia found in the usual form of this disorder. In this patient, with a male chromatin pattern, the morphology of the left gonad was identical with that found in gonadal dysgenesis, and on this side there occurred some differentiation of Müllerian duct derivatives represented by a Fallopian tube and a rudimentary uterus and vagina. On the right side, where the gonad had differentiated sufficiently to contain a few seminiferous tubules, a vas deferens was present. Apparently this testis in early fetal life had produced sufficient secretion to cause development of Wolffian duct derivatives on the same side, and partial masculine differentiation of the phallus and the urogenital sinus. We considered this patient to be a chromosomal male with a form of failure of male sex differentiation, transitional between that seen in gonadal dysgenesis and the classic forms of male pseudohermaphrodism. Because of the presence of immature seminiferous tubules in the right gonad and the absence of any female germinal elements, we have chosen to classify this patient as a male pseudohermaphrodite.

DISCUSSION

Aside from the practical diagnostic implications of the finding of a male chromatin pattern in 20 of a group of 22 patients with gonadal dysgenesis, the results of this study serve to corroborate some of the theories of human sex differentiation. Furthermore, the 2 patients reported in detail represent a link between gonadal dysgenesis in chromosomal males and the classic forms of male pseudohermaphrodism. These observations provide a spectrum of ambisexual development in chromosomal males which facilitates classification and suggests a mechanism for the anatomic variation in genital structures which may be observed in disturbances of embryonic sex differentiation.

Before exploring these considerations further, it is of value to analyze

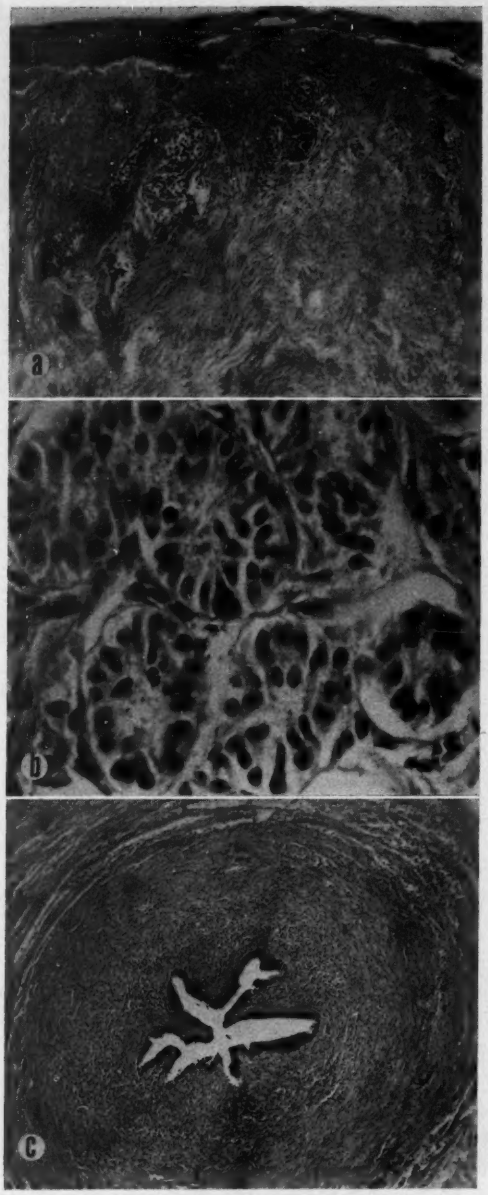

FIG. 7. The right gonad and genital duct in Case 2 (G.C.) **a.** Low-power photomicrograph of the right gonad (H & E; ×90), showing a small group of immature seminiferous tubules and the wavy gonadal stroma. Except for a few small clusters of seminiferous tubules, the appearance of the right gonad was identical to that of the left. **b.** High-power view of the immature seminiferous tubules (H & E; ×450). **c.** The appearance of the vas deferens on the right side (H & E; ×60).

briefly the normal pattern of intrauterine sex development in order to understand better the pathologic derangements which may occur.

Human Sex Development

In the normal course of human sex differentiation there is a series of events which transforms the embryo from a potentially bisexual organism equipped with gonadal and genital primordia capable of developing in a

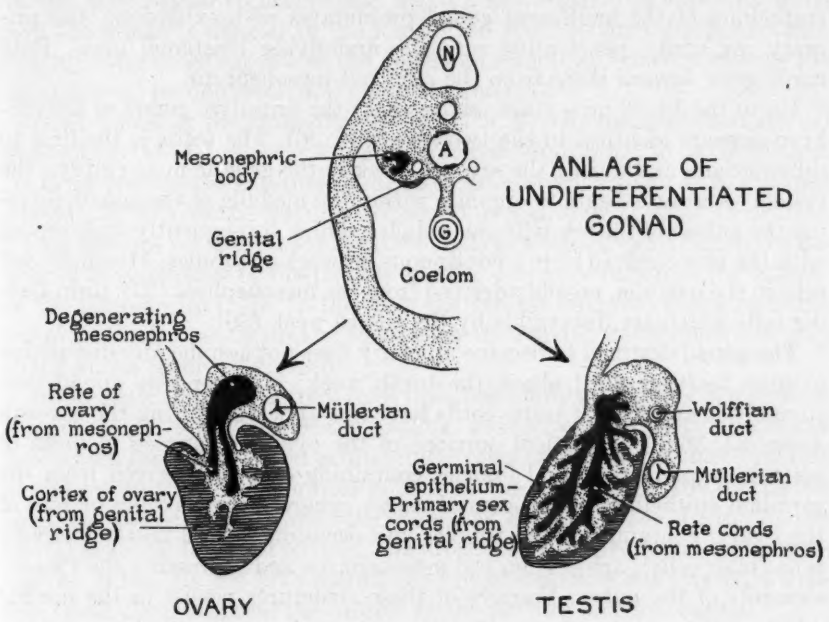

FIG. 8. A diagrammatic representation of the embryogenesis of the human ovary and testis, illustrating the anlage of the differentiating gonad. Note the predominantly cortical development of the ovary as compared with the medullary development of the testis. The relative contribution of components derived from the germinal epithelium (lined area) and the mesonephros (black area) is shown.

male or female direction, to a fetus with either normal male or female genital organs. In the differentiation of sex structures from the indifferent stage, the gonad is first affected, then the genital ducts, and finally the urogenital sinus and external genitalia. Normally, the development of all the sexual structures conforms to the chromosomal sex established in the zygote at the time of fertilization.

The differentiation of the gonads. By the fifth week of embryonic life (5–8 mm. stage) a bilateral longitudinal ridge (urogenital ridge) is present

along the dorsal mesentery protruding into the coelom. The ventromedian surface composes the genital ridge which lies adjacent to the mesonephros (Fig. 8). The surface is covered by a thickening of the coelomic epithelium —the germinal epithelium. This epithelium contains the primordial germ cells which are generally believed to have arisen from an extra-gonadal site, probably the yolk-sac entoderm, and migrated to the genital ridge early in embryonic life (33). Both the germinal epithelium and the mesonephros contribute to the development of the ovary and testis (33). The germinal epithelium of the indifferent gonad proliferates with extensions, the primary sex cords, penetrating into the underlying blastemal mass. Rete cords grow toward these from the adjacent mesonephros.

Up to the 14–16 mm. stage (sixth week) the primitive gonad of the embryo appears identical in the two sexes (34, 36). The testis is the first to differentiate, and during the seventh week in the genetic male embryo the primary sex cords begin to organize within the medulla of the gonad, forming the anlage of the seminiferous tubules, which subsequently anastomose with the rete cords to form a continuous network of tubules. Mesenchymal cells in the medulla, possibly derived from the mesonephros (33), form Leydig cells which are discernible by the eighth week (36).

The gonad destined to become an ovary does not assume any distinctive ovarian features until about the tenth week; however, the gonad presumably is an ovary if testis cords have not appeared during the seventh week (33, 36). The cortical portions of the ovary, composed of nests of epithelium and primordial follicles containing ova, are derived from the germinal epithelium of the genital ridges, whereas the medulla or rete of the ovary, containing small tubules and occasional small nests of Leydig cells (hilar cells), arises from the mesonephros and represents the "male" elements of the ovary. Vestiges of these structures persist in the normal adult ovary.

In the development of the testis the medulla or mesonephric elements predominate. In the ovary the reverse is true. The relative amount of tissue contributed by the germinal epithelium of the genital ridge far exceeds that arising from the mesonephros, and the ovary develops and persists primarily as a cortical gland. This is diagrammatically illustrated in Figure 8. Thus both ovaries and testis may be considered to have "male" (medullary) and "female" (cortical) components (33).

Differentiation of the genital ducts. In the embryo, during the indifferent stage, a paired set of genital ducts develops—the Wolffian or mesonephric ducts and the Müllerian ducts or oviducts. The Wolffian duct develops from the upper system of mesonephric ducts and tubules and is well differentiated by the fifth week (8 mm. stage). The Müllerian duct originates later from a groove in the urogenital ridge and first appears at nearly six

weeks (10 mm. stage). By the eighth week (25 mm. stage) both sets of ducts are well developed in the embryo (34, 35).

Although the embryo possesses a male and female set of duct primordia, the ducts themselves are not ambisexual. The Wolffian ducts are capable of differentiating into the epididymus, vas deferens and seminal vesicles and the Müllerian ducts of developing to form the Fallopian tubes, uterus and upper vagina. Beginning early in the third month the Müllerian duct in the male begins to degenerate, leaving as vestiges the appendix testis and the prostatic utricle. In the female the Wolffian duct disappears at a slightly later stage except for the vestiginal structures which persist as the epoöphoron, paroöphoron and Gartner's duct (35).

Development of the external genitalia[4]*:* The development of the external genitalia is the last phase of embryonic sex differentiation to be completed. At three months the external genitalia of the embryo usually have attained sufficiently distinctive features to permit recognition as male or female. The difference between the male and female genitalia depends upon the degree of development of the phallus and the extent of fusion in the midline of the labioscrotal folds. In the male the genital tubercle continues to develop to form a penile organ, whereas in the female, development is only slight and a clitoris is formed.

In the male the labio-scrotal folds fuse, beginning posteriorly and extending anteriorly to the base of the phallus. This covers the urogenital sulcus into which the urethral and genital ducts open and results in the formation of the scrotum and the perineal portion of the urethra. By the fourteenth week the penile urethra has fused as far as the glans (34). In the female the labioscrotal folds fail to fuse. The urogenital sulcus remains open, forming the vulva. By the fifth month the urogenital sinus has formed a shallow vestibule containing the orifices of the urethra and the vagina.

Gonadal Dysgenesis

Embryology of gonadal dysgenesis. In patients with gonadal dysgenesis the components of the gonad derived from the germinal epithelium of the genital ridges fail to develop. Nevertheless, varying amounts of mesonephric vestiges can be identified in the medullary region of these poorly differentiated gonads and in the mesosalpinx, indicating that there is not true agenesis, but rather a dysgenesis. This is well illustrated in our Case 1 (H.G.), the patient with gonadal dysgenesis and an enlarged genital tubercle (phallus), in whom masses of Leydig cells and mesonephric tubules were found.

That primordial germ cells are not essential for the differentiation of a

[4] The differentiation of the urogenital sinus is discussed elsewhere (34, 35, 37).

gonad has been well established in lower forms (38). However, absence of the germinal epithelium, due either to developmental failure or arrest, appears to result in a severe disturbance of gonadal differentiation in which the vestigial gonads of both sexes have a similar appearance. The architecture of the gonadal stroma in gonadal dysgenesis closely resembles that of the ovary but it is now apparent that this connective tissue is not sex specific.

Bearing on theories of human sex differentiation. There is general agreement that the differentiation of the primordial gonad into a testis or an ovary is established by the balance of an undetermined number of antagonistic genic determiners carried by both the sex chromosomes and autosomes (35, 38, 39). The possible modifying role in man of hormones or inductor substances present in the embryonic environment on *gonadal* differentiation is not known. Recently, Burns (40) reported the production of ovotestes in male opossum fetuses by the administration of estrogen during a critical period of gonadal morphogenesis—the first experimental demonstration of a hormonally induced gonadal inversion in mammals. The freemartin is the only evidence that hormonal factors may affect gonadal differentiation in placental mammals (41).

Conversely, there is a considerable diversity of opinion among experimental embryologists concerning the factors causing the differentiation of the *genital ducts* and the *external genitalia* in vertebrates. Moore and his group (42) believe that the genetic or constitutional pattern laid down in the zygote is the prime and sole determiner of future sex development in the embryo. Others (43, 44) are firmly convinced that hormones or inductor substances originating in the fetal gonad or from an extragonadal source evoke subsequent differentiation of the genital ducts and the external genitalia.

The first substantial evidence of the influence of the fetal gonad and its secretion on sex differentiation came from the classic study of "Nature's experiment," the bovine freemartin (a sexually abnormal female[5] twin of a male calf) by Lillie (41) almost forty years ago. From the interpretation of the freemartin arose the widely accepted theory that the gonad of each genetic sex produced a homologous hormone which governed the differentiation and growth of the genital tract in conformity with the gonad-ovary or testis, as the case might be. In addition the fetal gonadal secretion was believed to induce the regression of the heterologous duct. Accordingly, since sex differentiation was considered to be determined by different embryonic gonadal hormones in the two sexes, this concept became known as the *dihormonic* theory.

[5] Moore, Graham and Barr recently reported a female chromatin pattern in bovine freemartins (45).

With the advent of pure androgenic and estrogenic steroids, numerous investigators studied the influence of sex hormones on the development of the genital ducts and other accessory sex structures. These experiments have been extensively reviewed in the English literature by Burns (43), Greene (44), Moore (42) and Price (46), and in the proceedings of a recent international symposium (47). Of importance to this discussion is the failure of androgens or estrogens to influence significantly the regression of the heterologous genital ducts in placental mammals. Certain paradoxical effects also have been observed, such as the stimulation under some circumstances of the heterologous as well as the homologous set of genital ducts by either androgens or estrogens. This confusing effect has been attributed by Burns to the dosage and time of hormone administration and can be avoided by using more physiologic doses (43).

In 1935 Wiesner (48) took exception to the dihormonic theory. Predicated on studies in postnatally gonadectomized rats, he postulated that only the secretion of the fetal testis was of prime importance in sex differentiation, and this viewpoint became known as the *monohormonic* theory. However, not until the critical and brilliant experiments of Jost (1) on the effects of prenatal castration in the rabbit did this theory, as later modified by Jost, receive material substantiation.

Jost reported the results of a series of experiments (1, 49, 50) on the effects of intrauterine castration of rabbit fetuses at successive stages of sex differentiation, beginning at a stage when the testes could be identified histologically but the primordial genital tract (genital ducts, urogenital sinus, and external genitalia) was still undifferentiated and indifferent. The castrated fetuses were returned to the uterus and the animals sacrificed shortly before expected parturition. These studies demonstrated that castration of female fetuses did not prevent essentially normal differentiation of the female genital system, although in the youngest castrates the Müllerian derivatives were slightly reduced in size. However, removal of the fetal testes at an early stage resulted in male fetuses with entirely female development of the genital ducts, urogenital sinus and external genitalia, morphologically comparable to the early female castrates. Evidence obtained from study of chromosomal sex in patients with gonadal dysgenesis suggests that they are the human counterpart of the fetal rabbits castrated at an early stage in the experiments of Jost. The anatomy of the genital tract in fetuses castrated at an early stage is compared with the findings in typical cases of gonadal (ovarian and testicular) dysgenesis in Table 3. Similar results from early castration have been obtained in mice (2) and in the chick embryo (51).

By castration of fetal rabbits at successively later stages, Jost further demonstrated that the fetal testis exerted its masculinizing morphogenetic

TABLE 3. GENITAL STRUCTURES IN GONADAL (OVARIAN AND TESTICULAR) DYSGENESIS AS COMPARED WITH RESULTS OF EARLY FETAL CASTRATION IN THE RABBIT (JOST)

	Rabbit		Gonadal dysgenesis	
Chromosomal sex	♀	♂	♀	♂
Gonad	gonadectomized prior to day 20		germinal elements: 0 mesonephric elements: + to + +	
Genital ducts	♀	♀ (Wolffian derivatives absent)	♀	+ (Wolffian derivatives absent)
Urogenital sinus	♀	♀	♀	♀
External genitalia	♀	♀	♀	♀

effect over a rather short, critical period (1, 49, 50). During this period in male fetuses the subsequent development of first the genital ducts and later the urogenital sinus and external genitalia became fixed or stabilized for the remainder of fetal life, presumably by a testicular secretion. Castration at one-day intervals[6] during this critical phase resulted in a progressively more masculine appearing genital system until finally castration of male fetuses did not prevent essentially normal male sex differentiation. Wells and Fralich have made comparable observations in fetal rats (52). Table 4 contrasts the effects of castration of male rabbit fetuses at an intermediate stage during this period with the findings in a case (H.G.) of gonadal dysgenesis with phallic enlargement.

The findings in both chromosomal males and females with typical gonadal dysgenesis agree with the fetal castration experiments in demonstrating that female differentiation of the genital ducts and external genitalia always occurs in the absence of fetal testes. Similarly, these patients exemplify the essential and primary role of the testis in human embryonic sex development. Furthermore, that differentiation of a female genital system in the female or castrated male fetus does not require ovarian hormones or the influence of maternal or extra-gonadal feminizing substances, is suggested by two observations: 1) Cultivation *in vitro* of Wolffian and Müllerian duct primordia of the rat (53) and the chick (54) from either male or female embryos, in the absence of hormones, resulted in continued

[6] The average length of pregnancy in the rabbit is thirty days. The effects of castration were studied by removing the fetal gonads at one-day intervals between the nineteenth and twenty-fourth days.

TABLE 4. EFFECTS OF LATER FETAL CASTRATION IN THE MALE RABBIT (JOST) COMPARED WITH THE FINDINGS IN A CASE OF GONADAL (TESTICULAR) DYSGENESIS WITH PHALLIC ENLARGEMENT

	Rabbit	Case 1 (H.G.)
Chromosomal sex	♂	♂
Gonad	gonadectomized on day 22–23	germinal elements: 0 mesonephric elements: +++; abundant Leydig cells
Genital ducts	♀ (± ♂)	♀ (± ♂)
External genitalia	hypospadic ♂	ambiguous: phallus, separate vaginal and urethral orifice

growth of the Müllerian ducts while the Wolffian duct primordia atrophied. 2) Anterior-chamber grafts of Müllerian duct tissue obtained from male and female guinea pig embryos continued to differentiate when implanted in normal adult or spayed female guinea pigs (55).

There is also evidence from the effects of unilateral castration of rabbit fetuses that the morphogenetic secretion of the fetal testis migrates largely

TABLE 5. COMPARISON OF THE EFFECT OF UNILATERAL CASTRATION IN THE MALE RABBIT FETUS (JOST) WITH THE FINDINGS IN CASE 2

	Rabbit		Case 2 (G.C.)	
Chromosomal sex	♂		♂	
Gonad	Unilateral castration prior to day 21		Rt: Germinal elements: + (male); mesonephric elements: +++	Lt.: germinal elements: 0 mesonephric elements: +++
	Intact side (testis)	Castrated side		
Genital	♂	♀ (± ♂)	♂	♀ (± ♂)
Urogenital sinus	♂		♂	
External genitalia	♂		hypospadic ♂	

unilaterally along the genital tract, causing both the development of the Wolffian and regression of the Müllerian ducts (1, 50). Table 5 shows the similarity between the results of unilateral castration in the rabbit fetus and the abnormalities of genital development found in our Case 2 (G.C.). In this patient there was predominantly female development on the left

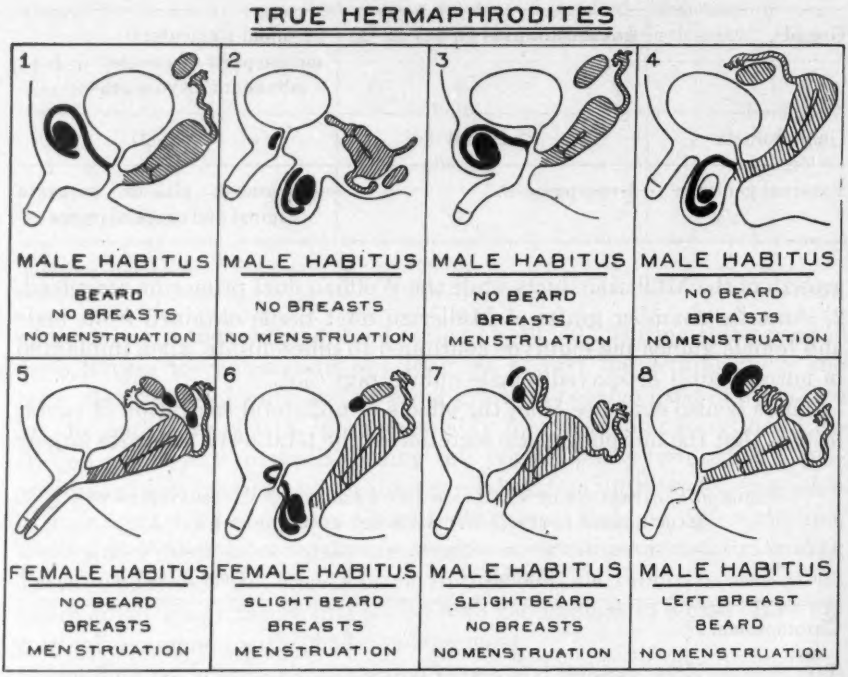

FIG. 9. The relationship between the gonads and the development of the genital ducts in 8 true hermaphrodites. Testicular structure and Wolffian derivatives are shown in black and ovarian tissue and Müllerian derivatives by cross-hatching. In the patients in the top row (Nos. 1–4) with an ovary and a testis on contralateral sides, the development of the genital ducts on the ipsilateral side followed that of the gonad (ovary or testis). When ovotestes were present on one or both sides (Nos. 5–8), predominantly Müllerian development of the genital ducts occurred. (Adapted from Wilkins (3).)

side where no germinal elements were present in the rudimentary gonad. On the right side, the primitive testicular structure was sufficient to cause differentiation of the Wolffian duct and partial masculinization of the external genitalia. Additional support for the unilateral action of the fetal testicular secretion is obtained from the group of true hermaphrodites, in

whom an ovary is present on one side and a testis on the opposite side (Fig. 9). In all such instances the development of the homolateral genital duct was compatible with that of the homolateral gonad, whereas the appearance of the external genitalia varied. This suggests that early in human sex development the testicular secretion primarily has a local action, at least as far as the genital ducts are concerned.

Little is known of the nature of the fetal testicular secretion (50). Although it is generally considered to be an androgen derived, primarily, from the fetal Leydig cells (50), its effects are not entirely identical to those

TABLE 6. DETERMINANTS OF HUMAN SEX DIFFERENTIATION

STRUCTURE	DETERMINED BY
1) *Gonad:* testis or ovary	1) *Genetic pattern:* sex chromosomes and autosomes
2) *Genital ducts:* Wolffian or Müllerian derivatives	2) *Gonad:* a) agonadal—always female (Müllerian) b) fetal testis—male (Wolffian)
3) *Lower genital tract:* *Derivatives of urogenital sinus* including caudal vagina and prostate *External genitalia* urogenital sulcus; labioscrotal fusion; phallic size	3) *Gonad* (? under influence of gonadotropin): a) agonadal—always female b) fetal testis—male

of testosterone or other known androgens. In placental mammals the injection of testosterone will cause development of the Wolffian ducts but will not cause retrogression of the Müllerian ducts. In the rabbit there is evidence that the secretion of the fetal testis is in part under the influence of the fetal pituitary (49, 50).

Normally the fetal adrenal does not seem to play a significant role in sex differentiation. However, in female pseudohermaphrodism due to congenital adrenal hyperplasia, the masculinization of the urogenital sinus and the external genitalia is caused by androgens produced prenatally by the pathologic adrenal (56). It is of interest that, despite the considerable masculinization of the lower genital tract in these individuals, the development of the genital ducts is entirely feminine.

A summary of genetic and gonadal factors determining sex differentia-

tion in humans is presented in Table 6. Our knowledge of other influences capable of modifying the normal pattern of sex development is meager, *e.g.*, the effects of maternal vitamin-A deficiency on the genital tract of the fetal rat observed by Wilson and Warkany (57). Much remains to be clarified.

Relationship of gonadal dysgenesis to male pseudohermaphrodism: Chromosomal males with the usual type of gonadal (testicular) dysgenesis should be regarded as the most severe and extreme form of male pseudohermaphrodism,[7] that is, they are genetic males who are anatomically completely feminized. In these individuals the absence of fetal testicular function is so complete that it affects the subsequent two stages of sex differentiation

TABLE 7. CLASSIFICATION OF AMBISEXUAL DEVELOPMENT IN CHROMOSOMAL MALES.* THE EFFECTS OF VARYING DEGREES OF FETAL TESTICULAR INSUFFICIENCY

A—*Gonadal Dysgenesis in Chromosomal Males*
1) Typical—simulating female.
2) With phallic enlargement.

B—*Classic Male Pseudohermaphrodism*
1) Simulant female with "feminizing testes"** and Wolffian vestiges.†
2) External genitalia ambiguous or resembling those of mlae.‡
   a) Müllerian duct derivatives predominant.
   b) Wolffian duct derivatives predominant.

C—*Hypospadic Males with Bifid Scrotum*†

* True hermaphrodites are not included in this classification, although some have a male chromatin pattern (10).

** Morris (58).

† Testes intra-abdominal or inguinal.

‡ Phallus resembles penis; usually hypospadias and cryptorchidism; occasionally penile urethra, separate vaginal orifice or urogenital sinus.

and the individual is born with the genital ducts and genitalia of a female. In patients similar to H.G. (Case 1); in whom mesonephric rudiments with Leydig cells are better developed, enlargement of the genital tubercle occurs as a result of masculinizing secretions. In G.C. (Case 2), the male pseudohermaphrodite with Müllerian vestiges and a hypospadic phallus, fetal testicular function on the right side was sufficient to initiate male sex differentiation but not to complete it.

Table 7 shows the relationship of chromosomal males with gonadal dysgenesis to pseudohermaphrodites with morphologically male gonads

[7] Without exception, a male chromatin pattern was found in all cases of male pseudohermaphrodism (verified by gonadal biopsy) studied by Barr (10) and the authors.

who have ambisexual differentiation of their genital ducts and/or external genitalia. In these cases it is necessary to postulate that there must have been a deficiency of fetal testicular function at some stage during the critical period of sex differentiation. The time and degree of the deficiency would determine to some extent whether the abnormalities involve principally the genital ducts or the external genitalia. That the secretory activity of the testis may not correspond to its morphologic structure is demonstrated by the fact that in a large group of male pseudohermaphrodites with female external genitalia, breast development and other estrogenic manifestations occur at puberty (58). There is also a possibility that in some of this group, the sexual hair, voice and external genitalia are unresponsive to androgen (59). In other types of male pseudohermaphrodites, either masculinization or feminization may occur at puberty but the testes almost invariably show faulty spermatogenesis and are sterile.

Pathogenesis of gonadal dysgenesis: Few disorders present such a multiplicity and variety of congenital anomalies as may be found in patients with gonadal dysgenesis (*see* Table 1, (60)). Multiple congenital malformations, particularly when symmetrical, have long been attributed to inheritable mutant genes, but only relatively recently has it been recognized generally that deleterious environmental influences may account for noninheritable, but multiple defects (61). There is little evidence that gonadal dysgenesis is genetically determined. The authors are not aware of an authentic case occurring in more than one generation or in more than one individual in a family, although isolated congenital defects often associated with this syndrome, such as webbed neck, may be familial. The gonadal defect precludes reproduction, but no associated anomalies were found in siblings or parents of affected individuals in over 50 cases seen in this Clinic. It is true, however, that a genetic etiology has been clearly established in some cases of male pseudohermaphrodism (62). Accordingly, until further data are available, a genetically determined defect with pleiotropic manifestations cannot be excluded.

The most likely basis for the syndrome would seem to be a deleterious agent of unknown nature, which adversely affects the embryo or the germ plasm. To affect sex differentiation in such a striking manner, the disturbance must occur before the eighth to ninth week in the male embryo and perhaps slightly later in the female. This time span is compatible with a developmental disturbance capable of producing many of the associated malformations. Some additional support for this hypothesis is obtained from the high incidence of low birth weights in affected full-term infants, the variability of the phenotype, and the superficial resemblance of some of the malformations which may be encountered to those seen as a result of fetal rubella infection (61), *e.g.*, cataracts, deafness and cardiac defects.

The high incidence of chromosomal males in patients with gonadal dysgenesis was an unexpected finding. Twenty of our patients were chromosomal males and only 2, chromosomal females. Polani *et al.* studied 3 cases and found a male pattern in all (12). The presence of coarctation of the aorta, relatively rare as an isolated defect in the female, led these workers to investigate the sex chromatin pattern. Barr and his associates (26) also observed a predominantly male sex ratio in an additional group of patients; 10 patients had a male chromatin pattern and 2 a female pattern. The fetal testis embryologically differentiates before the ovary (33, 34, 36) and the preponderance of chromosomal males suggests that the male gonad, particularly its germinal elements, may be more susceptible to damage from some unknown cause in early intrauterine life than the fetal ovary.

Relationship to Turner's syndrome in the male and to anorchia: Prunty *et al.* (63) have recently reviewed a group of 9 individuals with dwarfing, webbed neck, and other congenital anomalies but with normally formed male sex organs. Three of these patients had cryptorchidism and 5 had an elevated titer of urinary gonadotropin. Because of the similarities of habitus and congenital anomalies to those found in the syndrome of "ovarian agenesis," they have been reported to represent the male analogue of this syndrome. Our findings indicate that the chromosomal male with gonadal dysgenesis is the counterpart of this disorder in the chromosomal female. Although cases of Turner's syndrome in boys may show hypogonadism and maldeveloped testes, there is hypoplasia of testicular components and not dysgenesis with absence of germinal elements. In these patients, the testes during early fetal life were capable of bringing about male sex differentiation. Females have been described who had the typical habitus and various congenital anomalies associated with gonadal dysgenesis and who developed some secondary female characteristics at puberty. They would appear to represent the female counterpart of Turner's syndrome in boys.

Rarely, normally formed males are encountered in whom testes cannot be found after careful surgical exploration. The vas deferens ends blindly or terminates in a rudimentary epididymis. Masculinization does not occur at puberty. In these patients it would seem that testes must have been present during early fetal life and degenerated sometime after sex differentiation had been completed, possibly during migration into and through the inguinal canals.

Diagnostic value of determining chromosomal sex in gonadal dysgenesis: Previously, unless exploratory laparotomy or peritoneoscopy was performed, the diagnosis of gonadal dysgenesis could be made only by finding an elevated titer of urinary gonadotropins (FSH). In this laboratory high titers of FSH have not been observed in this syndrome before 9–13 years

of age; in only 2 instances was FSH demonstrable by the age of 7 (a significant finding in a sexually infantile child). In younger children, who proved later to have the syndrome, FSH could not be detected. Strong presumptive evidence of the diagnosis can now be obtained at any age in suspected individuals by finding a male chromatin pattern; a female pattern does not exclude the diagnosis. The determination of chromosomal sex by the skin biopsy technique is a valuable diagnostic procedure in the study of apparent females who are stunted in growth or have congenital malformations frequently associated with gonadal dysgenesis—especially infants and young children with a peculiar habitus and localized edema of the extremities.

No special differences in habitus or in responsiveness to estrogen have been correlated with the chromosomal sex pattern. The sexual orientation has been entirely feminine, irrespective of the chromosomal pattern, in all patients with gonadal dysgenesis studied in this Clinic by Hampson and associates (63). This has indicated that gender, including erotic orientation, is not automatically determined by the sex chromosomes nor by the sex hormones (32). The authors consider it is most important that the patients and their families should not be informed concerning their chromosomal sex when a male chromatin pattern is found, in view of present-day misconceptions of the importance of chromosomes in determining psychosexual outlook.

SUMMARY

1. Twenty of 22 patients with so-called "ovarian agenesis" have been found to be chromosomal males. Because of this observation and the histologic appearance of the vestigial gonads, the name *gonadal dysgenesis* is advocated.

2. A case of *gonadal dysgenesis with phallic enlargement*, and a case of unusual male pseudohermaphrodism are described, representing links between gonadal (testicular) dysgenesis and the classic varieties of male pseudohermaphrodism. The anatomic findings are comparable to those in fetal castration experiments in animals at various stages of embryonic sex differentiation. This indicates the essential role of the fetal testis in human sex development, and explains the female-type genital system found in chromosomal males with gonadal (testicular) dysgenesis.

3. A classification of ambisexual development in chromosomal males is presented, based on varying degrees of fetal testicular insufficiency. Chromosomal males with gonadal dysgenesis should be regarded as the most severe and extreme form of male pseudohermaphrodism.

4. In 5 patients under 6 years of age, the finding of a male chromatin pattern established the diagnosis of gonadal dysgenesis at an age, in our

experience, before urinary gonadotropin can be detected. A female chromatin pattern does not exclude the diagnosis at any age.

5. It is suggested that gonadal dysgenesis is due to a deleterious agent affecting the embryo before the eighth to ninth week of gestation.

6. The differences between gonadal dysgenesis, Turner's syndrome in boys, and anorchia are discussed.

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Addendum

Moore and Barr (65) and Marberger, Boccabella and Nelson (66) have described a simplified method for the detection of chromosomal sex, using smears from the oral mucosa.

Since this paper was submitted, it has been noted that an article on chromosomal sex and gonadal dysgenesis was published by a group of Brazilian workers in 1954 (Décourt *et al.* (67)). They reported 4 cases—2 with male type nuclei and 2 with female type nuclei.

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THE METABOLIC EFFECTS OF PROGESTERONE IN MAN*†

RICHARD L. LANDAU, M.D., DELBERT M. BERGENSTAL, M.D., KATHLEEN LUGIBIHL, A.B. AND MARY E. KASCHT, A.B.

The Department of Medicine, University of Chicago, Chicago 37, Illinois

INTRODUCTION

INTEREST in the metabolic effects of progesterone has heretofore centered primarily about its role in the reproductive functions of the female organism, where its restricted growth-promoting influence on the uterus and breast have been well documented (1, 2). Its more general metabolic properties have not been so extensively explored. In this category progesterone has been shown to be life-preserving and salt-retaining in adrenalectomized rats, ferrets and dogs (3–6) and salt-retaining in normal dogs when administered in 10–20 mg. doses per day (5, 6). It failed, however, to support the work capacity of adrenalectomized rats (7), and in a man with Addison's disease who was maintained with added salt, 30 mg. of progesterone per day had no influence on urinary chloride excretion (8). Recently it has been demonstrated that progesterone induces growth in the sebaceous glands of rats (9). In contrast to this report of an anabolic effect and similar influence on the uterus and breast is the report of Abels and Dobriner (10) that 100 mg. daily of progesterone is catabolic in a normal man. This brief description of a single metabolic experiment seems to have been overlooked, or perhaps discounted as the record of a freak or toxic reaction. Prior to the present study (11), Abels and Dobriner's results had not been confirmed.

It was the intent of this investigation to determine the metabolic consequences of approximately physiologic amounts of progesterone in man. As the amount of pregnanediol excreted daily during the luteal phase of the menstrual cycle is from 2–7 mg. (12), and there are several estimates to the effect that as a rule 10–20 per cent of parenterally administered progesterone is excreted as urinary pregnanediol (13), it may be estimated

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that the amount of progesterone secreted during the luteal phase of the menstrual cycle in from 10–70 mg. per day. In early pregnancy, during which the excretion of pregnanediol ranges from 5 to 40 mg. per day (14), 50 to 400 mg. of progesterone may be delivered to the organism daily. We accordingly selected 50 and 100 mg. of progesterone per day as dosages which might approximate the maximum rate of secretion during the normal menstrual cycle and would be well within the range of secretion during pregnancy.

The response to progesterone was determined in several patients with intact and presumably normal adrenal glands. Progesterone was also given to 1 woman and 2 men with Addison's disease who were receiving replacement quantities of desoxycorticosterone and cortisone, and to a third man with Addison's disease both before and during hormone replacement. Thus it was possible to obtain information concerning the influence of adrenal hormones and of adrenal secretory activity on the metabolic effects of progesterone.

METHODS

These investigations were carried out in the Metabolic Unit, where patients were maintained on constant regimens in the usual manner. Changes induced in the metabolic status of the subjects were defined by significant shifts from a control series of determinations of various urinary constituents. Thus, when values rose above such a control baseline a loss was recorded; and when they dropped below the control baselines, retention was said to have occurred. Determinations were always perfomed on 24-hour or 48-hour pools. By comparison with 4-day or 6-day collections, these relatively short collections are felt to enhance the sensitivity and the precision of such studies. Complete balances were not carried out but, on occasion, estimates of the total nitrogen balance were made by assuming a constant fecal nitrogen of about 1 gram daily (15). Completeness of urine collections was ascertained by creatinine determinations.

All of the progesterone[1] used in these studies fulfilled U.S.P. specifications for purity. Melting-point specifications were met. The trace of contaminants to be found in such a preparation would not be expected to be metabolically active. The progesterone was dissolved in sesame oil and 20 per cent benzyl benzoate and administered by intramuscular injection. The 50-mg. daily dose was given in a single 1-ml. injection; the 100-mg. daily dose was administered in two injections of 50 mg. each, given about ten hours apart. In the patients with Addison's disease the cortisone acetate in aqueous suspension was given in one intramuscular injection each

[1] Progesterone generously supplied by Dr. Edward Henderson of Schering Corporation, Bloomfield, N. J.

day, and the desoxycorticosterone acetate dissolved in sesame oil in one daily intramuscular injection.

Methods for the determinations of total urinary nitrogen, inorganic phosphorus, creatine, creatinine, uric acid, alpha amino acids, sodium, potassium, chloride and 17-ketosteroids have been previously detailed (16). Pregnanediol was determined according to the procedure of Stimmel (17). The technique of Randolph (18) was followed for eosinophil counts. Urinary urea was estimated by means of the Conway micro-diffusion technique (19); and blood urea by the same Conway procedure and by means of a modification of Ormsby's method (20). The same method for blood urea was employed throughout each study.

PROTOCOLS

M.K. (Fig. 1) was a 23-year-old girl with no apparent organic disease. Her basal metabolic rate was −17 per cent[2]; basal calories were 1,250 per twenty-four hours.

J.S. (Fig. 2) was a 47-year-old man with a chronic, mild to moderately severe rheumatoid arthritis. His metabolic rate was −12 per cent, with basal calories of 1,600 per twenty-four hours.

M.P. (Fig. 3) was a 36-year-old woman who was moderately obese and had experienced menstrual irregularities for twelve years. She had been amenorrheic for eighteen months. Her metabolic rate was −19 per cent; her basal calories were 1,160 per twenty-four hours. She menstruated on withdrawal of the progesterone.

X.C. (Fig. 4) was a 41-year-old woman with rather inactive rheumatoid spondylitis. Her metabolic rate was −15 per cent, with basal calories of 1,120 per twenty-four hours.

V.W. (Fig. 5) was a 36-year-old woman with Addison's disease. She was treated throughout with 50 mg. of cortisone acetate and 3 mg. of desoxycorticosterone acetate per day. Her metabolic rate was ±0; basal calories were 1,500 per twenty-four hours. She felt sick toward the end of the period of progesterone administration, and on the last day of treatment an adrenal crisis was diagnosed. The serum sodium concentration had dropped from 137 to 132 mEq. per liter.

V.V. (Fig. 6) was a 42-year-old laborer with Addison's disease. He received 2 mg. of desoxycorticosterone acetate and 25 mg. of cortisone acetate every day. His metabolic rate was −10 per cent; basal calories were 1,370 per twenty-four hours. The serum sodium concentration declined from 138 to 128 mEq. per liter as a result of the progesterone treatment.

J.B. (Fig. 7) was a 26-year-old man with newly discovered Addison's disease. He had recently lost 20 pounds and was emaciated. His metabolic rate was −20 per cent, with a basal caloric requirement of 1,420 per twenty-four hours.

H.C. (Fig. 8) was a 40-year-old Chinese plant foreman. The diagnosis of Addison's disease had been made about two weeks before this investigation was begun. He was just well enough to get along for a limited time in the hospital with only 8 grams of added salt per day as treatment. The effects of progesterone were accordingly studied while he was maintained on the salt alone and again while he was receiving, in addition, 25 mg. of cortisone acetate and 1 mg. of desoxycorticosterone acetate daily. His ultimate replacement requirement on leaving the hospital was 37.5 mg. of cortisone per day and

[2] Basal metabolic rates were calculated on the basis of Mayo Foundation standards.

2 mg. of desoxycorticosterone (buccal). The sodium intake as calculated from the known consumption of salt and the published estimates of the sodium content of foods was 241 milliequivalents per day. The baseline of 231 milliequivalents thus suggested that there was a slightly negative sodium balance before replacement therapy was started. The serum sodium concentration did not decrease from 135 mEq. per liter during the first course of progesterone, but dropped to 129 mEq. per liter just before cortisone and desoxycorticosterone were begun. The concentration then rose to 138–140 mEq. and subsequently fell to 135 mEq. per liter during the second progesterone course. His metabolic rate was −17 per cent, with basal calories of 1,170 per twenty-four hours.

RESULTS

The metabolic effects of progesterone in subjects with apparently normally functioning adrenal glands were sufficiently uniform to permit a description of the results as a group. In every instance 50 mg. of progesterone per day induced a rise in urinary nitrogen excretion which began during the first or second 48-hour pool and reached peak values 1.7–2.4 Gm. per day higher than control levels. In the representative studies detailed here the nitrogen elevations as measured from pretreatment baselines averaged 1.6 Gm. in M.K. (Fig. 1), 1.0 Gm. in J.S. (Fig. 2), 1.1 Gm. in M.P. (Fig. 3) and 1.1 Gm. during the first six days in X.C. (Fig. 4). In all subjects urinary nitrogen fell to the baseline level or below within four days after treatment was discontinued.

In J.S. the enhanced nitrogen excretion was maintained for the thirty days of treatment; and no abatement was noted during the sixteen and fourteen days of administration of progesterone in M.P. and M.K., respectively. In contrast, the urinary nitrogen elevation waned after six days in X.C. (Fig. 4) and equilibrium was restored during treatment with 50 mg. of progesterone daily. Doubling the dosage at this point did not increase the nitrogen excretion. After treatment was stopped, however, urinary nitrogen dropped to a level about 1.0 Gm. below the control average. Thus the influence of progesterone was sustained for thirty-two days, although a degree of counteraction may well have been exhibited. A similar counteracting influence was illustrated in 2 other subjects (data not shown here).

In M.K. (Fig. 1) the rise in urinary urea closely paralleled the rise in total nitrogen, in this instance accounting for virtually all of the increased nitrogen excretion. In all of the studies, fasting blood urea nitrogen concentrations were either uninfluenced or were elevated 1–3 mg. per 100 ml. during the period of enhanced nitrogen excretion. Accordingly the induced losses of urea could not be explained as a strictly renal effect and must indicate a net acceleration of protein catabolism.

Of the other nitrogenous constituents of urine determined, only uric acid excretion seemed to be increased (Figs. 1 and 3), and the increases

were very slight. Alpha amino-acid excretion did not rise, and endogenous creatinuria was depressed during progesterone treatment in X.C. (Fig. 4), during the first course in M.P. (Fig. 3), and in 2 other women (detailed data not presented). Creatine excretion was not affected in J.S. (Fig. 2), or during the second course in M.P. (Fig. 3).

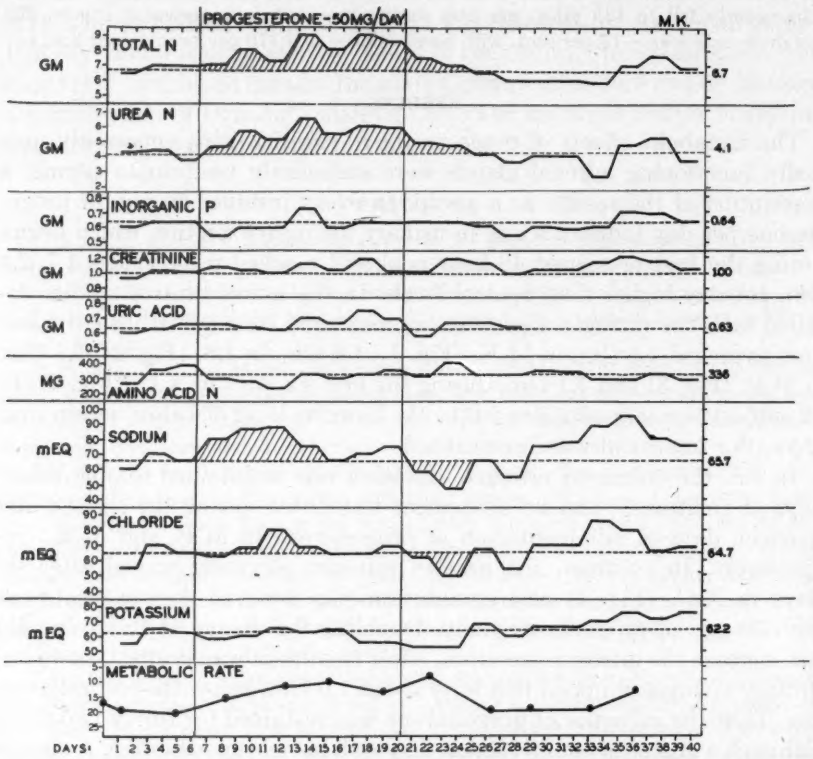

FIG. 1. The effect of progesterone administration in a young woman **M.K.**, on the excretion of several urinary constituents and on the basal metabolic rate. Horizontal broken lines indicate the averages of control values. The diet was 185 Gm. carbohydrate; 50 Gm. protein (nitrogen, 8.0); 120 Gm. fat; calories, 2,020.

The mildness of this catabolic response to progesterone is attested to, not only by the limited nitrogen losses, but also by the absence of parallel elevations in the urinary inorganic phosphorus and potassium during either 50-mg. or 100-mg. daily dosages. In M.P. (Fig. 3), increasing the progesterone to 100 mg. per day seemed to evoke a greater nitrogen loss, the elevation averaging 1.7 Gm. in comparison with the daily loss of 1.1 Gm.

induced by a dosage of 50 mg. In the 2 other subjects for whom data are not given, the same increase in dosage also led, at least temporarily, to an additional rise in nitrogen excretion. More detailed dose-response relationships have not been studied.

Uniformity also prevailed with respect to the influence of progesterone

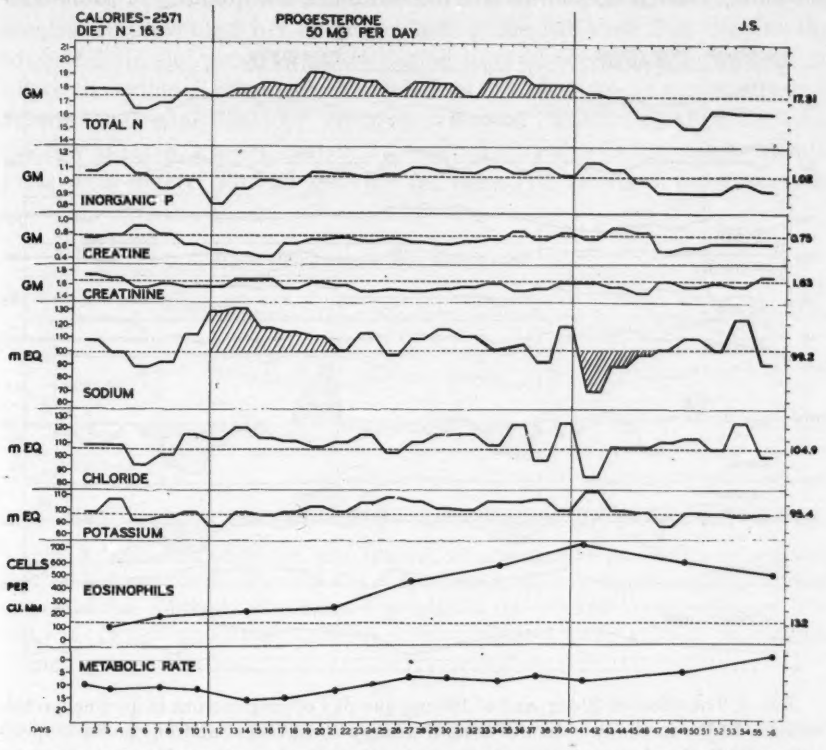

FIG. 2. The effect of progesterone administration in an arthritic man, **J.S.**, on the excretion of several urinary constituents, circulating eosinophils, and the basal metabolic rate. Broken horizontal lines indicate the averages of control values. The diet was 237 Gm. carbohydrate; 102 Gm. protein (nitrogen, 16.3); 135 Gm. fat; calories, 2,571.

on urinary sodium and chloride excretion in the subjects with functioning adrenals. The rather wide control variations which are not unusual in such experiments were observed in several subjects, but the consistent effects of progesterone were never masked by baseline irregularities. Sodium excretion always rose during the first two days of progesterone administration and then declined over several days, reaching the baseline before progesterone was discontinued. In J.S. (Fig. 2) this required ten days, and

in M.K. (Fig. 1) eight days. In M.P. (Fig. 3) the baseline excretion was resumed after the first two-day pool on the 50-mg. dose, and after four days when a 100 mg. per day was given. The increase in urinary sodium was also greater as a result of the larger amount of progesterone in the latter patient. In each case prompt compensatory sodium retention developed immediately after progesterone was discontinued, the quantity of sodium re-

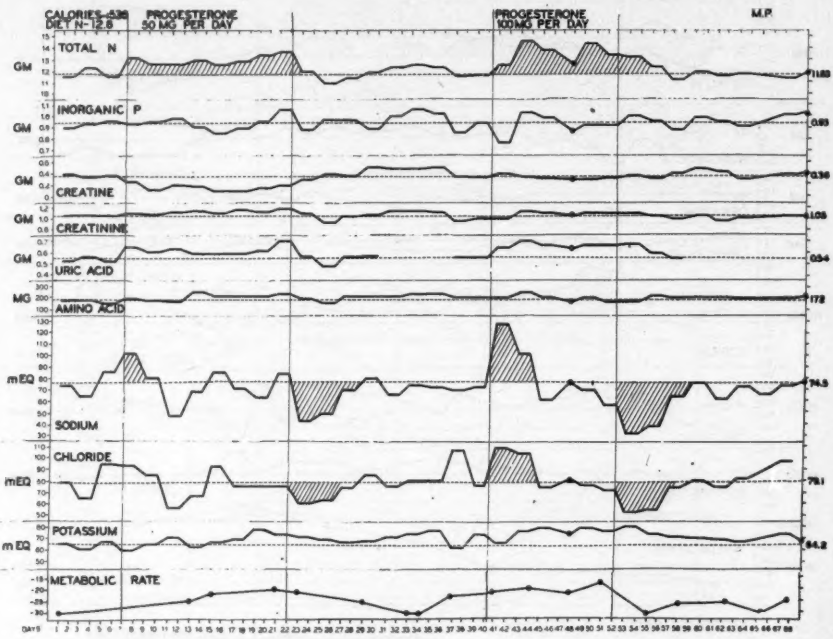

FIG. 3. The effect of 50 mg. and of 100 mg. per day of progesterone in an amenorrheic woman, **M.P.**, on the excretion of several urinary constituents and on the basal metabolic rate. Broken horizontal lines indicate the averages of pre-treatment control values. The diet consisted of 141 Gm. carbohydrate; 81 Gm. protein (nitrogen, 12.8); 72 Gm. fat; calories, 1,536.

tained approximating that previously lost. Urinary chloride excretion tended to parallel the foregoing shifts in sodium, but in all instances the movement of chloride was substantially less than that of sodium. The volumes of urine also tended to follow the trends in sodium excretion, but shifts in the output were never spectacular.

In patients with Addison's disease who were fully treated with both cortisone acetate and desoyxcorticosterone acetate the metabolic influence of progesterone was more intense and enduring, and the salt losses were

substantially greater than in normal subjects. The first of these subjects, V.W. (Fig. 5), received the most vigorous course of progesterone. Her urinary nitrogen excretion began to increase on the fifth day of treatment with 50 mg. per day, reached a level averaging 2.1 Gm. in excess of the control average, and then rose another gram when a dose of 100 mg. per day was given. The Addisonian crisis which developed on the last day of treatment prevented her from consuming the full diet, but despite the lower caloric and protein intakes during the two succeeding days, urinary nitrogen remained elevated for eight days after treatment was stopped. A spontaneous compensatory nitrogen retention, which averaged 1.6 Gm. per day at its maximum, ensued. In contradistinction to the results in subjects with active adrenal glands, the enhanced nitrogen excretion was

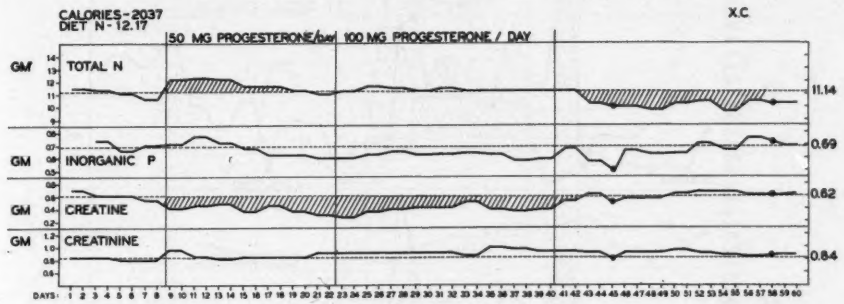

FIG. 4. The effect of 50 mg. and 100 mg. of progesterone per day in an arthritic woman, **X.C.**, on the urinary excretion of total nitrogen, inorganic phosphorus, creatine and creatinine. The broken horizontal lines indicate the averages of control values. The diet was 179 Gm. carbohydrate; 76 Gm. protein (nitrogen, 12.2); 113 Gm. fat; calories, 2,037.

paralleled by an elevation in urinary inorganic phosphorus, potassium and creatine.

Similar results were obtained in 2 men with Addison's disease. In V.V. (Fig. 6) there was a positive nitrogen balance of about 2 Gm. per day (dietary nitrogen, 13.3 Gm.; control urinary nitrogen, 10.3 Gm.) when pregesterone was started. On 50 mg. per day his total nitrogen excretion rose 4.2 Gm., and took eight days to return to a new baseline which approximated nitrogen equilibrium. Inorganic phosphorus and potassium were also lost during this catabolic phase. Urinary uric acid rose slightly. J.B. (Fig. 7), the third Addisonian, was recovering from the inanition of uncontrolled adrenal insufficiency and showed a positive nitrogen balance of approximately 6 Gm. per day when progesterone treatment was begun (dietary nitrogen, 19.2 Gm.; control urinary nitrogen, 12.3 Gm.). Despite

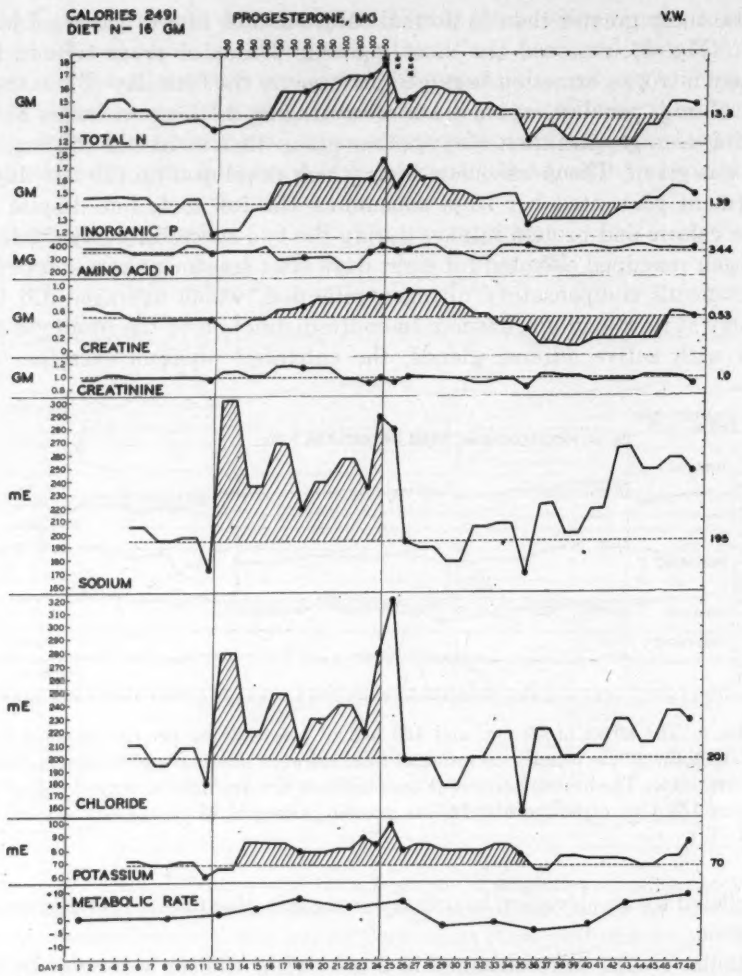

FIG. 5. The effect of 50 mg. and 100 mg. per day of progesterone in an Addisonian woman, **V. W.**, on the excretion of several urinary constituents, and the metabolic rate. Horizontal lines indicate the averages of control values. She received 50 mg. of cortisone acetate and 3 mg. of desoxycorticosterone acetate per day throughout. Her diet was 318 Gm. carbohydrate; 100 Gm. protein (nitrogen, 16.0); 153 Gm. fat; calories, 2,491.

* Normal saline, 500 ml. intravenously.

** Normal saline, 1,000 ml. intravenously. Dietary intake—146 Gm. carbohydrate; 65 Gm. protein; 73 Gm. fat; calories 1,501.

*** Dietary intake—204 Gm. carbohydrate; 93 Gm. protein; 123 Gm. fat; calories, 2,283.

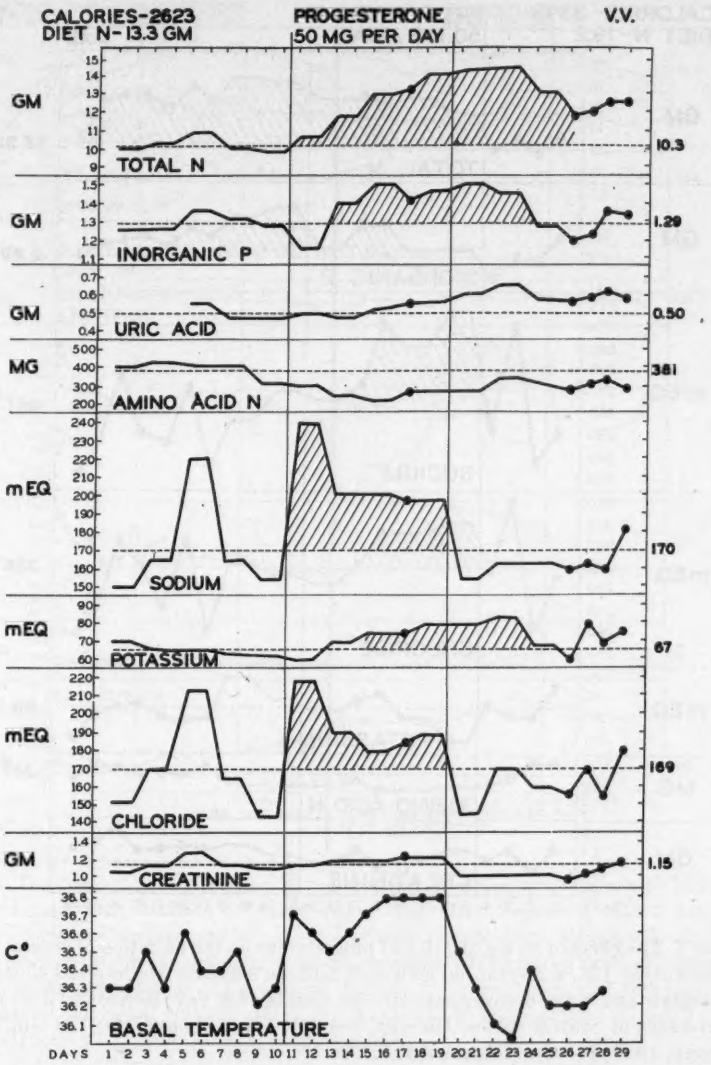

FIG. 6. The effect of 50 mg. per day of progesterone in a 42-year-old laborer with Addison's disease, **V.V.**, on the excretion of several urinary constituents and on the basal body temperature. He received 2 mg. of desoxycorticosterone acetate and 25 mg. of cortisone acetate per day throughout the study. Broken horizontal lines indicate the averages of control values. The diet was 278 Gm. carbohydrate; 83 Gm. protein (nitrogen, 13.3); 132 Gm. fat; calories, 2,623.

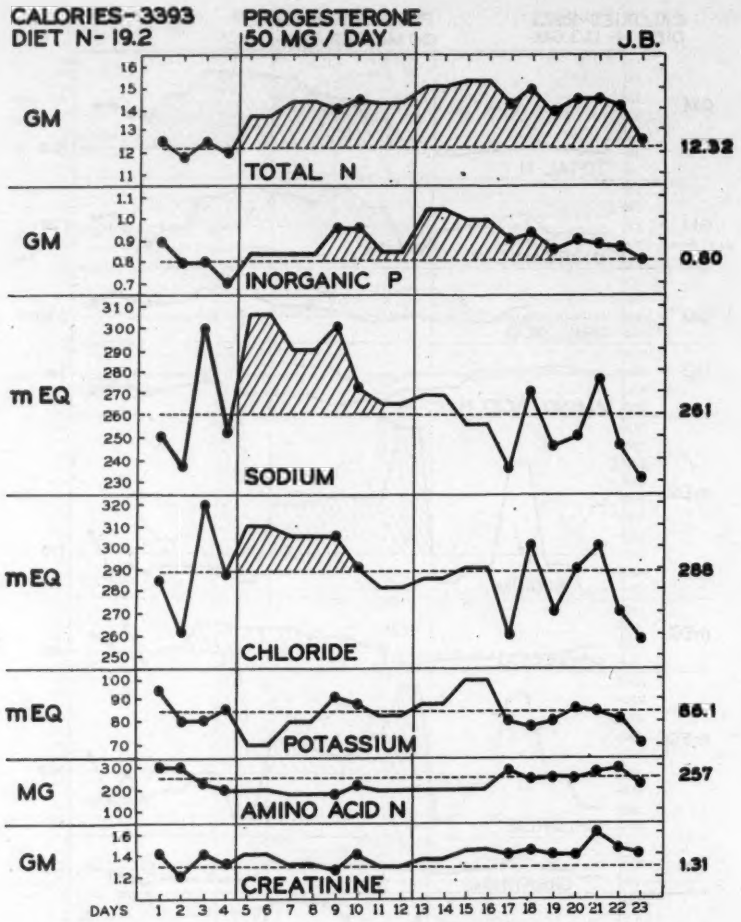

FIG. 7. The effect of 50 mg. per day of progesterone on the excretion of several urinary constituents in **J.B.**, a 26-year-old man with Addison's disease. He received 25 mg. cortisone acetate and 1 mg. desoxycorticosterone acetate each day. Horizontal lines indicate the averages of control values. His diet was 366 Gm. carbohydrate; 120 Gm. protein (nitrogen, 19.2); 161 Gm. fat; calories, 3,393.

this vigorous anabolic impulse, 50 mg. of progesterone per day led to an elevated nitrogen excretion which averaged 15.4 Gm., or 3.2 Gm. per day greater than the control value. Following treatment urinary nitrogen declined slowly, and required eleven days to return to its original level. As in the other Addisonians, inorganic phosphorus excretion was also enhanced, but potassium excretion was not affected in this subject.

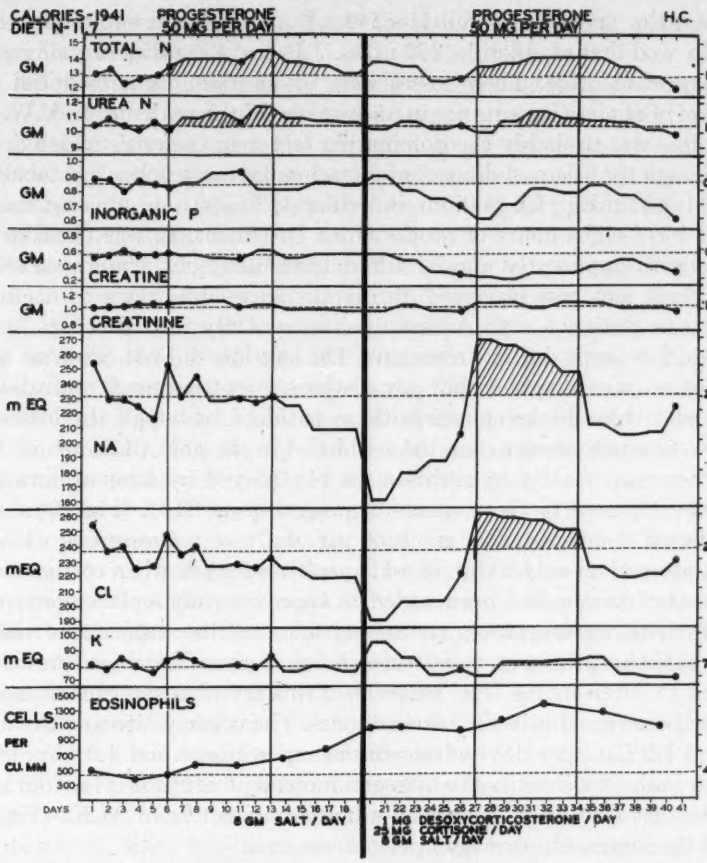

FIG. 8. The comparative effects of 50 mg. per day of progesterone given to **H.C.**, a 40-year-old man with Addison's disease, before and after treatment with 1 mg. of desoxycorticosterone acetate and 25 mg. of cortisone acetate daily, on the excretion of several urinary constituents and on circulating eosinophils. The heavy vertical line indicates the change in treatment of the adrenal deficiency; broken horizontal lines indicate the averages of the initial control values. His diet was 176 Gm. carbohydrate; 73 Gm. protein (nitrogen, 11.7); 105 Gm. fat; calories, 1,941.

Amino-acid nitrogen excretion was uninfluenced in all of the patients with adrenal deficiency.

The natriuresis and chloruresis induced in the hormone-treated Addisonians were dramatic, despite sizable control variations in sodium and chloride excretion. Accompanying increases in urine volume were moderate. In V.W. the urinary sodium loss (quantity in excess of the control average)

was 819 mEq. and the chloride loss 546 mEq. In V.V. the sodium loss was 342 mEq. and that of chloride, 207 mEq. J.B. lost 198 mEq. of sodium and 128 mEq. of chloride. These losses were unquestionably substantial and they were of clinical importance in at least one of the patients. In V.W. the sodium loss was probably the dominating factor in the crisis which developed, though the serum sodium concentration did not reach a spectacularly low level (132 mEq./L). Sodium and chloride losses were greatest during the first forty-eight hours of progesterone treatment, as was usual in the patients with apparently normal adrenal function; but a more sustained loss of these ions was observed during the succeeding days of declining excretion in patients with Addison's disease. Only in J.B. (Fig. 7) did compensation occur during treatment. The salt loss did not continue after progesterone was stopped, but no distinct post-treatment retention of sodium and chloride developed in these patients, although the losses incurred were much greater than those induced in the non-Addisonians.

In order more clearly to evaluate the role played by adrenal hormones in the development of these effects of progesterone, H.C. (Fig. 8), a man with adrenal deficiency, was given 50 mg. daily of progesterone while he was maintained on only 8 Gm. of added salt, and again when cortisone and desoxycorticosterone had been added in approximately replacement quantities. In both circumstances the magnitude and the duration of the induced catabolic processes were somewhat less than had been previously observed in Addisonians. The patterns of urinary nitrogen excretion were almost superimposable in the two instances. The urinary nitrogen elevation averaged 1.0 Gm. per day before steroid replacement and 1.0 Gm. afterward; in both experiments the nitrogen increment continued for four days after the last day of progesterone administration. As in M.K. (Fig. 1), most of the increase in urinary nitrogen was urea.

Initially, urinary inorganic phosphorus was uninfluenced by progesterone administration in H.C. (Fig. 8). The introduction of cortisone and desoxycorticosterone, however, was followed by a distinct and progressive lowering of phosphorus excretion. The second progesterone course then induced a rise in urinary phosphorus which coincided with that of nitrogen, and returned to the original low point after treatment. Urinary creatine was also unaffected by the first progesterone course. The spontaneous creatinuria, like inorganic phosphorus excretion, was suppressed by the introduction of cortisone and desoxycorticosterone treatment; subsequently, a questionable rise paralleled the foregoing increases in nitrogen and phosphorus. Urinary potassium was not noticeably influenced by either course of progesterone, although it rose as expected following the introduction of adrenal replacement therapy.

The influence of progesterone on urinary sodium and chloride was

strikingly different before and after the administration of cortisone and desoxycorticosterone. When H.C. was treated only with 8 Gm. of added salt, no effect was observed. The slowly diminishing course of excretion of these ions seemed undisturbed by the progesterone. Desoxycorticosterone and cortisone then caused the expected sharp retention of sodium and chloride. Following this, progesterone induced a loss of both ions (sodium 232 mEq.; chloride 160 mEq.) which was of the order previously noted in the other Addisonians.

The anticipated slight elevation in basal body temperature during treatment with progesterone was observed in every subject, but there was no fever. Metabolic rate determinations performed approximately twice a week in M.K., J.S., M.P., and the Addisonian V.W. (Figs. 1, 2, 3 and 5) furnished no conclusive evidence for a rise due to progesterone administration. In both M.P. and M.K. there might have been a slight increase in B.M.R. In M.P. (Fig. 3), the B.M.R. before progesterone was −29 per cent, and the peaks were −20 per cent on 50 mg. and −16 per cent on 100 mg. daily. In M.K. (Fig. 1) the controls averaged −17 per cent, the high point on progesterone was −10 per cent, and a value of −9 per cent was obtained two days after treatment. The slight malaise usually associated with progesterone therapy could have accounted for these questionably significant elevations. The very gradual and delayed upward trends in V.W. and J.S. could be explained by the developing crisis in V.W. and an increase in the severity of arthritic symptoms in J.S.

Circulating eosinophils were either uninfluenced, or there was a delayed and protracted increase in concentration as noted in J.S. and H.C. (Figs. 2 and 8). The rising eosinophil counts seem to have resulted from a reaction to the sesame oil and benzyl benzoate medium in which the progesterone was dissolved, since injections of the oil alone induced similar elevations in the eosinophil count in other subjects. However, the vehicle did not account for the metabolic effects described. Its administration to 2 arthritics (data not shown here) did not influence nitrogen metabolism or salt excretion, although progesterone dissolved in the same amount of oil evoked the usual catabolic response.

Urinary 17-ketosteroid excretion was not increased as a result of progestrone treatment. A systematic 3-mg. decline and subsequent recovery was observed in the young Addisonian, J.B. (Fig. 7), possibly reflecting a suppression of testicular androgen secretion.

The excretion of urinary pregnanediol during progesterone administration tended to be somewhat greater than anticipated in those subjects in whom it was determined. M.K. (Fig. 1) excreted 1.2 mg. daily prior to therapy and 16.2 mg. per day in the sixth two-day pool during treatment. The control level in M.P. (Fig.3) was 1.24 mg. In the last two days of the

50-mg. dosage period she excreted 12.9 mg. of pregnanediol daily, and in the next to the last pool, while receiving 100 mg. per day, the excretion was 22 mg. daily. J.S. (Fig. 2) excreted 11.5 mg. of pregnanediol daily in the middle of his course of 50 mg. of progesterone per day, and 13.3 mg. daily at the end of the course. The level of pregnanediol excretion in H.C. (Fig. 8) reached 7.7 mg. daily the last two days of his first series of injections. In X.C. (Fig. 4) the level rose to 8.1 mg. daily during treatment with 50 mg. of progesterone daily, and only reached 8.9 mg. before the discontinuance of the 100-mg. daily dosage of progesterone. The 50-mg. dosage thus produced pregnanediol elevations of 7 to 15 mg. per day. From these results it would appear that daily doses of 50 mg. of progesterone are probably slightly in excess of the maximum rate of progesterone secretion during the progestational phase of the menstrual cycle. However, the quantities of pregnanediol excreted indicate that 50 and 100 mg. daily are both within the range of the amount of progesterone secreted per day during pregnancy.

DISCUSSION

The administration of progesterone increased urinary nitrogen excretion in 12 subjects reported here, establishing that the hitherto isolated and unappreciated report of Abels and Dobriner (10) is truly representative. The benzyl-benzoate sesame-oil medium in which the progesterone was dissolved was metabolically inactive, although the eosinophilia occasionally seen after progesterone was reproduced by the oil alone. Minor local irritations at the sites of injection occurred only occasionally. Except for the 1 Addisonian in whom a crisis was induced by exaggerated metabolic effects, and 1 arthritic whose rheumatoid process was apparently intensified, the progesterone provoked very slight malaise or none at all. No fever was induced. The progesterone used had a high chemical standard of purity. Accordingly, although a description of the effects of this hormone as secreted is essential, the nitrogen dissipation so consistently seen here commands attention as a response which is probably of physiologic significance.

The large amounts of nitrogen lost in a long experiment suggest a rather general catabolic response. Although the daily nitrogen increments were never extreme, the accumulated loss from a protracted course of progesterone represented considerable protein destruction. For example, in J.S. (Fig. 2) in whom the increase in nitrogen excretion averaged just 1.0 Gm. daily, the aggregate loss of protein calculated from the nitrogen loss during the thirty days of treatment approximated 750 Gm. Such amounts could hardly be restricted to a single tissue or organ, with the exception of muscle.

The composition of the nitrogen loss offers no especial clue as to its source. The increments in urinary nitrogen consisted mainly of urea, indicating that the normal course of protein catabolism was followed. There was no increase in amino-acid excretion and urinary uric acid rose very slightly. Creatinuria was increased in only 1 subject, the Addisonian, V.W. (Fig. 5). Since a crisis was developing at the time, however, the creatinuria may have been secondary to the adrenal insufficiency itself rather than a primary effect of progesterone. The development of an enhanced creatine excretion only in this unusual circumstance cannot be accepted as evidence that muscle is a site of action of progesterone when nitrogen is lost.

Certain tissues may well be more or less selectively influenced by progesterone, just as the lymphoid tissues appear to be more sensitive to the catabolic influence of excesses of adrenal corticoids (21). In all likelihood the uterus, breast parenchyma and perhaps other tissues were spared here. However, no conclusions concerning the locus of the effect can be drawn from the data presented.

There was no evidence to suggest that the catabolic effect of progesterone was the result of stimulation of the adrenal or the thyroid. The slight elevations in uric acid excretion did not resemble the prompt and substantial rise in urinary uric acid characteristic of adrenal stimulation and no other indications of adrenal activation, such as a rise in urinary 17-ketosteroids (22), were observed. The response in Addisonians was even greater than in subjects with normal adrenals.

The elevations in basal metabolic rate that were occasionally seen were not always correlated with the loss of nitrogen, nor were they consistent enough or of sufficient magnitude to suggest activation of the thyroid gland. An increase in urinary creatine, which might suggest a thyroid effect, did not occur with regularity. Furthermore the failure of urinary alpha amino-acid nitrogen excretion to rise as part of the catabolic process distinguishes this response from the catabolism induced by adrenal and thyroid hormones (23). It is therefore likely that the acceleration of protein catabolism is a metabolic property of progesterone itself.

The data suggest a comment concerning the means by which progesterone accelerates protein catabolism. The rise in basal body temperature of 0.3–0.4 degrees Fahrenheit due to progesterone is probably too small to increase the basal metabolic rate detectably. However, calculations based on basal calories indicate that a portion of the nitrogen loss might be accounted for by the increased caloric requirement, provided the movement of fat into the metabolic pool was simultaneously inhibited by progesterone. For example in V.V. (Fig. 6), the basal calories of 1,370 per day would be augmented by 27 calories as a result of the temperature elevation. On a constant diet, 6.8 Gm. of protein must be mobilized to supply this energy.

The metabolism of this quantity of protein would produce an increment in urinary nitrogen slightly in excess of 1.0 Gm. Nitrogen excretion actually rose 4.2 Gm. in V.V. as a result of the administration of progesterone, and the elevation continued for six days after the basal temperature had returned to its initial level. Accordingly, only a portion of the catabolic effect could be accounted for by this mechanism, and other perhaps more direct processes for accelerating protein catabolism must be involved.

Although progesterone did not exert its catabolic force by acting through the adrenals, the presence of active adrenal glands did affect the character of the response. The catabolic process was generally more intense and enduring in the patients with adrenal deficiency. Nitrogen losses were greater in the Addisonians, and were accompanied by parallel increases in urinary inorganic phosphorus. Potassium excretion was also enhanced in 2 of these subjects.

Several explanations can be offered for the more limited catabolic effect of progesterone in subjects without adrenal defects. Although a final decision between alternatives cannot be made at this time, the matter is of sufficient importance to warrant outlining the possibilities here. The homeostatic influence of adrenal secretion could be exerted in four ways: 1) A stable quantity of corticoids may promote a situation conducive to the maintenance of nitrogen equilibrium without exerting a direct influence on protein metabolism (24). 2) Varying quantities of corticoids operating in an indrect fashion may be required for the optimum support of protein metabolism under different circumstances (24). 3) A direct protein-supporting effect may be exerted by an increased secretion of corticoids. 4) A specific anabolic secretion of the sort which has been previously proposed (25, 26) may be elaborated to counteract the progesterone effect. Of these, only the first suggestion does not involve an added secretion. If the catabolic effect of progesterone were opposed in such an indirect manner, the less intense catabolism induced in normal subjects could be explained by the greater effectiveness of native corticoids as compared with the cortisone and desoxycorticosterone used in treating the Addisonians. On the other hand the more rapid termination of the catabolic response after the discontinuance of progesterone in most of the subjects without adrenal disease could be an indication of the active participation of the adrenal glands in any of the manners suggested. The possibility that active adrenal opposition was generated in response to the progesterone received some support from the data on the effect of progesterone on sodium and chloride excretion.

The consistent sodium and chloride dissipating influence of progesterone came as a surprise, in view of its previously noted salt-retaining effects in intact dogs and in adrenalectomized dogs, ferrets and rats (3–6), and in

view of the absence of effect of progesterone on chloride excretion in an Addisonian treated with added salt (8). The apparent disagreement was reconciled in part by the observations made on H.C. (Fig. 8). Results of this study in an Addisonian demonstrated that the effect of progesterone on sodium and chloride excretion differed, depending on the presence or absence of circulating adrenal hormones. The characteristic salt loss was induced by progesterone while he was treated with desoxycorticosterone and cortisone, but when he was maintained on salt alone, sodium and chloride excretion was not influenced.

This difference in response between the treated and untreated Addisonian can be best explained as an inhibition of the salt-retaining influence of desoxycorticosterone and cortisone by progesterone. Presumably this interaction occurs in the kidney. The natriuresis and chloruresis induced by progesterone generally displayed the same pattern which has been reported to follow the withdrawal of adrenal secretions or of active adrenal preparations (27, 28). The peak excretion of sodium and chloride always occurred soon after progesterone treatment started, and the maximal excretion of these ions characteristically developed during the first few days of adrenal deficiency. In both circumstances the losses of sodium substantially exceeded those of chloride. This competitive type of reaction, so clearly demonstrated in the Addisonian H.C., would similarly account for the salt loss in subjects with intact adrenals in whom the progesterone opposed the intrinsic salt-retaining adrenal secretions. Thorn (29) has suggested a similar competitive reaction between cortisone and desoxycorticosterone.

Thus, in the doses employed, progesterone may have no influence on sodium and chloride excretion in the absence of adrenal secretions, and a salt-dissipating effect in the normal subject or in the hormone-treated adrenal-deficient human subject by virtue of a competitive inhibition of adrenal steroids. The salt-retaining influence of progesterone in intact dogs (5, 6) could be an effect peculiar to that species. However, large amounts of progesterone are also salt-retaining in adrenalectomized dogs, rats and ferrets. Much larger amounts of progesterone than those employed here might also be salt-retaining in man.

The influence of progesterone on sodium and chloride excretion was modified by the presence of active adrenals. Salt losses were less intense and of shorter duration in normal subjects than in the treated adrenal-deficient patients, and a compensatory retention of sodium and chloride after progesterone was discontinued developed only in the subjects with intact adrenal glands. The smaller losses of sodium and chloride induced in the non-Addisonians could be accounted for by a less effective inhibition of endogenous salt-retaining hormones by the administered progesterone. However, the post-treatment retention of salt in these individuals cannot

be so readily explained in this manner. In constantly maintained Addisonians, the lack of development of a sharp compensatory response despite greater salt losses would suggest that the permissive role of adrenal hormones cannot account for this protective reaction. Accordingly, the enhanced secretion of a salt-retaining adrenal steroid is postulated to explain this process. Such increased adrenal secretory activity could also account for the limitation of salt losses in the normal subjects. The particular secretion involved here may be aldosterone (30).

Although the dosages cannot be said to apply to all of the natural circumstances during which progesterone may be secreted, these results indicate that the physiologic influence of progesterone in man cannot be considered as being restricted to sex-linked structures. Androgen and estrogen, the other sex hormones, have previously been shown to possess general anabolic properties (31). The catabolic effect so consistently produced by progesterone in these studies places it, too, among the hormones which may play a role in the regulation of the growth process. This aspect is particularly important in pregnancy, when very large amounts of progesterone are secreted. Its role here in the face of the rapid growth of the fetus and certain maternal tissues is obscure. However, the possibility that it inhibits the general deposition of protein in the maternal organism in order to favor the growth of selected tissues and the fetus must be given serious consideration.

This demonstration that progesterone may competitively inhibit the salt-retaining influence of adrenal hormones and effect thereby a loss of sodium and chloride is also particularly pertinent in its relationship to pregnancy. In this manner progesterone could exert an inhibitory influence on the tendency of the pregnant organism to store salt and water.

These results also prompt a reconsideration of the role of progesterone in the physiology of the menstrual cycle. The progesterone secreted in the normal woman may exert an inhibitory influence on somatic growth and could also be expected to enter into the control of salt and water excretion. In these connections it is imperative that these studies be extended to include smaller dosages of progesterone.

SUMMARY

1. Approximately physiologic amounts of progesterone (50 to 100 mg. daily) exerted a mild to moderately intense catabolic influence in men and women.

2. The catabolic response was not mediated by the adrenal glands and there was no decisive evidence that thyroid activation resulted from progesterone administration. It was concluded that the accelerated protein catabolism was an effect of progesterone itself.

3. The nitrogen losses were usually greater in the patients with adrenal deficiency. In these subjects the enhanced nitrogen excretion was usually accompanied by an increase in urinary inorganic phosphorus, and in 2 cases urinary potassium was also elevated. The excretion of these constituents was not increased in non-Addisonians. Creatine retention accompanied progesterone treatment only in several normal subjects. These differences indicated that the catabolic effect of progesterone was opposed by adrenal secretions.

4. The same dosages of progesterone induced sharp rises in urinary sodium and chloride excretion, which were considerably greater in Addisonians treated with cortisone and desoxycorticosterone than in subjects with apparently normal adrenal function. The sodium losses exceeded those of chloride. Subjects with functioning adrenals retained sodium and chloride in the immediate post-treatment period.

5. In a patient with Addison's disease, progesterone induced no loss in urinary sodium and choride when given prior to hormone replacement therapy, and induced the usual natriuresis and chloruresis during treatment with cortisone and desoxycorticosterone. It was accordingly suggested that progesterone induced the salt loss by virtue of a peripheral (renal) competitive inhibition of salt-retaining corticoids.

6. The post-treatment retention of salt which developed only in normal subjects was suggestive of an enhanced secretion of salt-retaining corticoids. Such increased adrenal secretory activity could also explain the more limited salt losses in subjects without adrenal defects.

7. These metabolic effects of progesterone should be considered both in relation to the physiology of pregnancy and the menstrual cycle, and to the hormonal regulation of somatic growth.

Acknowledgment

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THE OCCURRENCE OF MONO- AND DI-IODOTYROSINE IN THE BLOOD OF A PATIENT WITH CONGENITAL GOITER*

JOHN B. STANBURY, M.D.,† A. A. H. KASSENAAR, PH.D., J. W. A. MEIJER AND J. TERPSTRA

The Division of Metabolic and Endocrine Diseases, Academisch Ziekenhuis, Leiden, The Netherlands

CONGENITAL enlargment of the thyroid gland is occasionally seen in areas where goiter is not endemic. Although the patient usually carries the stigmata of hypothyroidism, past or present, the thyroid gland itself may have an unusually strong affinity for iodide (1, 2, 3). Oftentimes several members of the same family are afflicted. Pathologic examination of thyroid tissue usually has disclosed intense hyperplasia of the follicular epithelium, and sometimes cystic degeneration and fibrosis.

The present report concerns a patient who had a progressively enlarging thyroid gland from birth. In addition to small amounts of thyroxine and tri-iodothyronine, he was secreting mono- and di-iodotyrosine into his blood.

CASE REPORT

The patient (Fig. 1) was a 27-year-old unmarried clerk who was born in a village near Utrecht, Holland, which was known as a region where goiter was common at that time. He had lived in Den Haag for the previous twenty-five years. Delivery was said to have been normal, but enlargement of the thyroid gland was noticed at birth. At nine months of age daily medication with 50 to 75 milligrams of thyroid powder was begun and continued in various dosages.

Early development and dentition were slow. Puberty occurred in his sixteenth year. He did well in school, learned several languages, and finished secondary education. He was always slow physically. Eight years before entry, and again three years before entry he was hospitalized for prolonged periods for pulmonary tuberculosis. Thyroid medication was discontinued five years before entry because of the fear that it might activate his tuberculosis. It was then observed that the thyroid began to enlarge in size. He was referred to the hospital by Dr. Peter Blom because of progressive compression of the trachea and esophagus.

His parents were school teachers and were unrelated. Neither had a goiter. He had one normal older sibling, and one brother aged 25 who had a goiter at birth. The latter brother was not given thyroid powder until several years after birth. His development

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† On a Fulbright Grant. Usual address: Massachusetts General Hospital, Boston, Massachusetts.

was retarded. With special instruction he was able to learn simple reading and writing.

At the time of admission the patient's height was 183 cm., span 181½ cm., and weight 81 Kg. His pulse was normal and his blood pressure was 110 systolic and 85 diastolic. His voice was low and rasping, and movements were slow. He was of normal intelligence. He complained only of slight difficulty in swallowing and of cold sensitivity. Pubic and axillary hair were sparse, and there was temporal recession of the hair line. The skin was smooth and slightly cool. The thyroid gland was large and multilobular. There was no

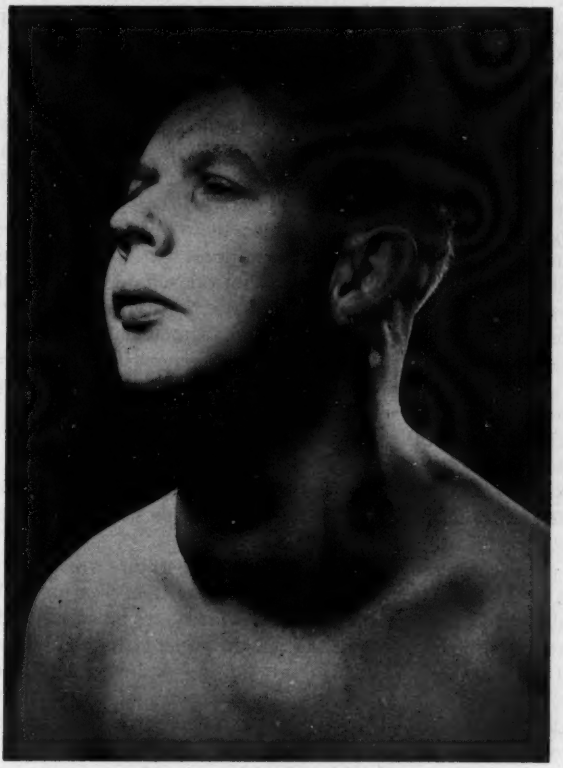

FIG. 1. A 27-year-old male with an enlarging thyroid gland. Goiter was noticed at birth.

thrill or bruit. The testes were smaller and softer than normal. There was normal relaxation of the tendon reflexes.

Examination of the blood disclosed a hemoglobin of 74 per cent, a red blood cell count of 3.8 million per cu. mm., and normal white cell and differential counts. Urinalysis findings were normal. The basal metabolic rate was −29 per cent. The protein-bound iodine concentration of the serum was 0.5 microgram per 100 ml. The blood cholesterol concentration was 380 mg. per 100 ml. Assay of urinary gonadotropins was positive for 6 mouse units and negative for 90. Results of serum assay for thyrotropic hormone were within the limits of normal for the method (4). Values for blood electrolytes, a water

tolerance test, and an insulin tolerance test were within normal limits. His skeletal age was 18 years. X-ray study of the chest disclosed minimal pulmonary tuberculosis which was probably inactive.

A diagnosis of congenital goiter with hypothyroidism was made. At the completion of the studies described here the gland was surgically removed. Convalescence was uneventful.

METHODS

Measurement of the accumulation and retention of I^{131} was made with a Geiger-Müller tube placed 30 cm. from the manubrial notch. A correction was made for the I^{131} in the extrathyroidal tissues by taking measurements from the thigh. A correction was also made for backscatter. Urine samples were measured in a liquid counting Geiger-Müller tube, as described by Veall (5). Samples of blood serum were assayed in a well-type scintillation crystal detector. Serum precipitable I^{131} was determined in the same tubes without transfer after precipitating with 10 per cent trichloroacetic acid and washing three or four times with 1 per cent trichloroacetic acid.

Serum for chromatographic analysis was brought to a pH of 2.0 with sulfuric acid. Samples measuring 1–6 ml. were extracted three times with *n*-butanol which had been brought to equilibrium with half saturated sodium thiosulfate. The pooled butanol extract was neutralized with ammonia and evaporated to dryness in an air stream. The residue was taken up in a small quantity of methanol-ammonia and placed on Whatman No. 1 filter paper strips. After initial analyses had disclosed I^{131} at the positions on the paper usually occupied by mono- and di-iodotyrosine, carrier quantities of approximately 80 micrograms of mono- and di-iodotyrosine were routinely added to the paper. Similar quantities of tri-iodothyronine and thyroxine were also added. A sample of normal serum to which I^{131} was added was extracted in an identical way: no detectable I^{131} was found at the mono- or di-iodotyrosine position on the chromatogram. Similarly, there was no detectable labeled mono- or di-iodotyrosine when labeled thyroxine and iodide were initially added to normal serum.

Descending phase chromatography at constant temperature was employed. For most of the analyses a butanol-dioxane-ammonia solvent system was used (6), and about twenty hours allowed for the separation. In a few instances an acetic acid-butanol system was used (6). Upon completion of the run the paper strips were dried and stained with diazotized sulfanilic acid. The strips were cut in ½ or 1 cm. lengths and counted in the well-type scintillation crystal detector. The geometry of the detector was such that the counting rate of a paper slip differed by no more than 2½ per cent from that when the slip was eluted with alkali.

At the time of surgical removal of the thyroid, representative sections were taken for histologic analysis, and the remainder was homogenized. Approximately 1 gram of the homogenate was digested with dehydrated pancreatic powder[1] for three days. Chromatographic analysis of an *n*-butanol extract of the digest was performed as previously described in a butanol-dioxane-ammonia system. To a measured amount of the butanol extract 25 mg. of *d-l*-thyroxine was added. After evaporation of the butanol the residue was dissolved in 25 per cent ammonia and the thyroxine was precipitated as the free acid by the addition of 10 per cent acetic acid. This precipitation of the thyroxine fraction with acid was repeated five times. After this, the thyroxine was recrystallized four times as the potassium salt. Constant specific activity was reached in the second crystal-

[1] Generously supplied by Dr. Rosalind Pitt-Rivers.

lization (7). From the specific activity of this material and from that in the initial butanol extract to which the thyroxine was added, the percentage of I^{131} bound in thyroxine and present in the butanol extract of the gland could be calculated.

RESULTS

In vivo *and urinary excretion curves.*

The *in vivo* uptake and retention curves and the urinary excretion curves shown in Figure 2 were obtained after an oral tracer dose of 50 μc. of I^{131}

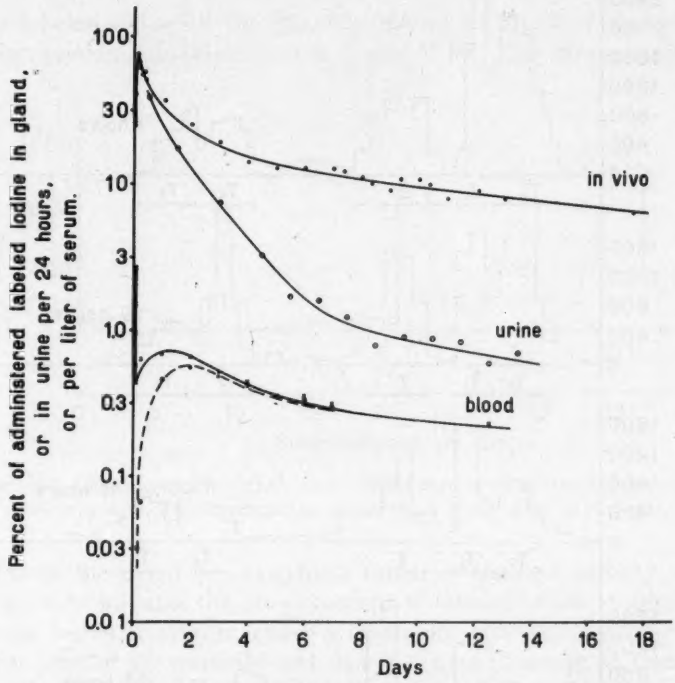

FIG. 2. The fate of labeled iodine in the patient of Figure 1. The tracer dose of I^{131} was given at zero time. The abscissa is in days. The ordinate is in percentage of administered dose of I^{131} in the gland (*in vivo*), or excreted per day in the urine, or per liter of serum. The solid line of the serum curve is total labeled iodine; the dashed line is the trichloroacetic-acid precipitable labeled iodine. Note that the scale of the ordinate is logarithmic.

This isotope accumulated rapidly in the gland and reached a maximum observed value of 74 per cent of the administered dose within two and a half hours. Subsequently there was a rapid release of labeled iodine from the gland so that at twenty-four hours 36 per cent remained in the gland,

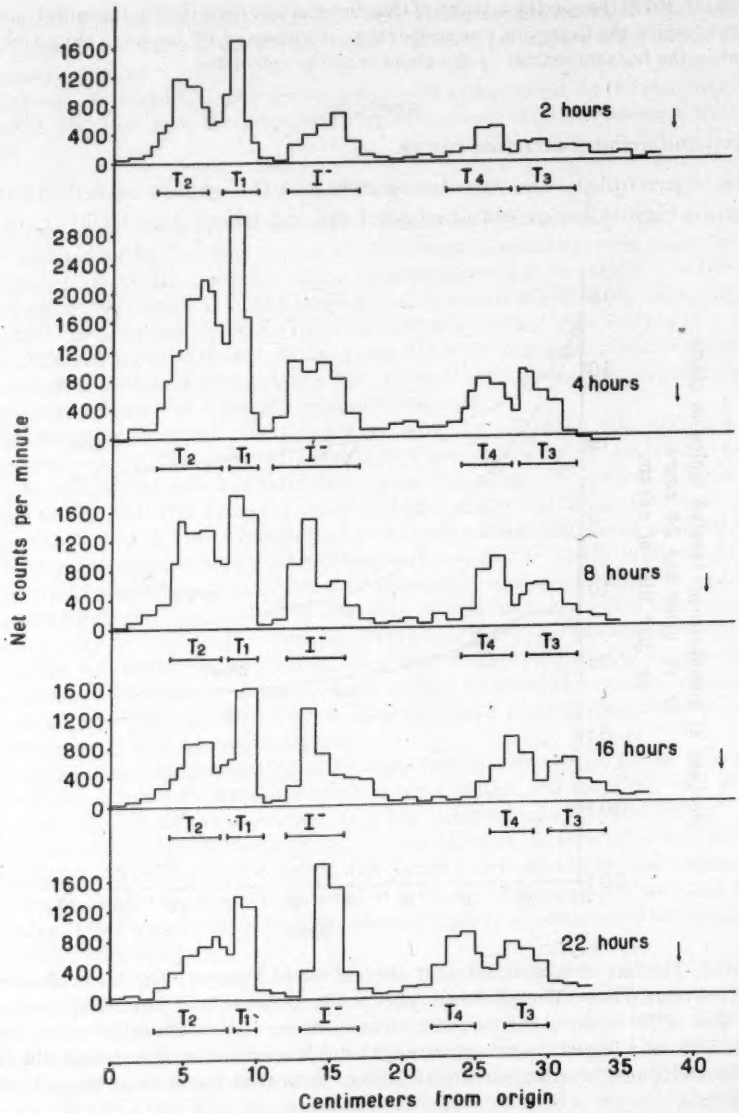

FIG. 3. The labeled components of the serum at serial times after administration of I^{131}. The abscissa is in centimeters from the origin of the chromatograms. The solvent front is indicated by an arrow. The ordinate is in net counts per centimeter of the paper strip; 1000 counts per centimeter represent approximately 0.02 per cent of the administered dose per liter of serum. T_1 = mono-iodotyrosine; T_2 = di-iodotyrosine; T_3 = triiodothyronine; T_4 = thyroxine; I^- = iodide. The solvent system was butanol-dioxane-ammonia. The spots were located with added carriers, as described in the text.

and at forty-eight hours only 25 per cent. After this time the fall was less precipitous, as may be seen in the figure. Of the administered dose, 36 per cent appeared in the urine in the first twenty-four hours, and 53 per cent in the first forty-eight hours. After seven days the urine curve continued to fall at about the same rate as the curve of thyroidal retention. The daily excretion of I^{127} for five successive days varied from 41 to 73 micrograms, with a mean of 55.

Serum curve

The labeled iodine of the blood as shown in Figure 2 was measured after intravenous administration of 1 mc. of I^{131}. The disappearance rate

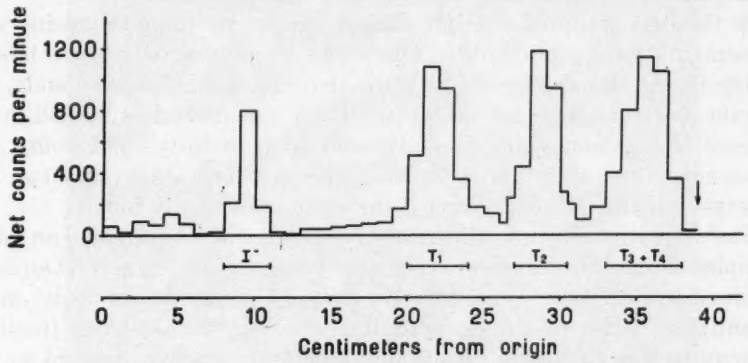

FIG. 4. Chromatographic analysis of pooled sera in an acetic acid-butanol solvent system. The construction of the chart is the same as in Figure 3.

of I^{131} from the blood was extremely rapid for the first seventy minutes. After seventy minutes the blood content of labeled iodine stopped falling and then began to rise, reaching a maximum observed concentration of 0.68 per cent of the administered dose per liter of serum at twenty-four hours. The fraction of the labeled iodine of the serum which was precipitable is also shown in Figure 2. It may be seen that during the rising phase of the blood curve a large fraction of the total labeled iodine was not precipitable. Thus labeled iodine, either as iodide or as a nonprecipitable compound, was returning to the blood within two hours after the initial dose of labeled iodide was given. The subsequent course of the serum curve was similar to that of the *in vivo* and urine curves.

Chromatographic studies

Chromatographic analyses were performed in a butanol-dioxane-ammonia system on 4 blood samples taken daily for four days after the second

tracer of I^{131}, and in duplicate on 5 samples taken during the 22-hour period between an oral dose of 2 mc. of I^{131} and surgical removal of the thyroid gland. Chromatographic analysis of 2 pooled samples of serum in a butanol-acetic acid system was also performed. In all samples mono- and di-iodotyrosine were demonstrated, except that these substances could not be found with certainty in the 72-hour and 96-hour samples. When carriers were added, as was usually the case, the I^{131} corresponded to within $\frac{1}{2}$ cm. of the stained carriers, as may be seen in Figures 3 and 4. When carriers were not added, the position of the labeled iodine corresponded to the positions of carriers run in parallel control strips. There was no significant amount of labeled iodine at the origin. The level of radioactivity was insufficient to permit radioautography of the paper strips.

In the first group of 4 serum samples mono- and di-iodotyrosine were present at twenty-four hours. There was proportionately more labeled iodine in the di-iodotyrosine than in the mono-iodotyrosine bands, but specific activities were not estimated. There was much less labeled iodine present in the mono- and di-iodotyrosine spots at forty-eight hours, and these substances were not detectable with certainty at seventy-two and ninety-six hours, although labeled thyroxine was readily found.

The 5 chromatograms illustrated in Figure 3 were made on blood samples drawn during the twenty-two hours before surgery. Duplicate chromatograms were qualitatively identical and showed only minor quantitative differences in the percentage recovery in the various fractions. It may be seen that both mono- and di-iodotyrosine were present at two hours. The concentration was increased at four hours, and subsequently declined. A surprising finding was the early appearance of thyroxine and tri-iodothyronine. Quantitative data on concentrations in the various peaks are not presented: reliable values could not be calculated because the total recovery in the five peaks of the chromatograms was only 40 to 54 per cent of the total labeled iodine of the blood samples from which the chromatograms were derived.

Analysis of thyroid gland

Eighty per cent of the I^{131} present in the crude tryptic digest of the gland was extractable into *n*-butanol. It was demonstrated by isotope dilution performed in this extract that 3.4 per cent was recoverable as thyroxine. As estimated from the chromatogram, an approximately equal amount was tri-iodothyronine. The remainder consisted of a small amount of iodide and large amounts of mono- and di-iodotyrosine. The gland content of I^{127} calculated from the concentration in the homogenate, was 3.25 mg.

Pathology of the thyroid

The removed gland weighed 198 Gm. It was multinodular. Most of the nodules on cut section were fleshy in appearnace, but some areas showed cystic degeneration and others had the appearance of colloid goiter. Microscopic study disclosed a varied pattern. Some areas resembled colloid goiter, with large collections of colloid in well formed follicles with low epithelium. Others showed masses of cells in no regular pattern, without colloid, but with fibrosis. Most areas showed small follicles with high

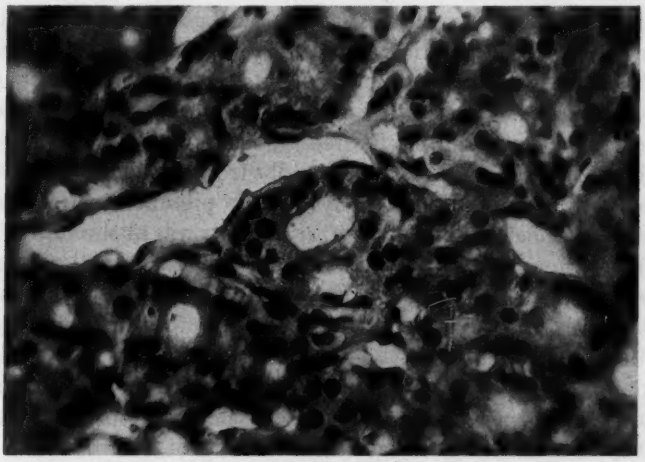

FIG. 5. Histologic pattern of thyroid tissue removed at operation. This was the predominant structure, but other areas showed more tendency to form colloid, and in some regions cystic degeneration and fibrosis were observed.

cuboidal epithelium and little or no colloid formation. This last pattern is illustrated in Figure 5.

Thiocyanate study

The brother of the patient was available for a study of the effect of KSCN on the retention of accumulated iodide by the thyroid. One hour after oral administration of the I^{131}, maximal retention of the isotope was observed in the gland. One and a half hours later, while the retention curve was essentially flat, he was given 2 Gm. of KSCN in water. No significant change occurred in the counting rate over the neck for the next two and a half hours of observation. The gland contained 77 per cent of the administered dose of I^{131} at five hours.

DISCUSSION

The enlargement of the thyroid glands of patients with congenital nonendemic goiter presumably is a compensatory effort in response to defective synthesis of thyroid hormone. Speculatively one might surmise that congenital goiter would occur in any patient when there is a failure of any one of the steps in the complex chain of reactions which lead to the final elaboration of the thyroid hormone. Two types of defect have already been distinguished. The thyroid glands of one group are incapable of incorporating accumulated iodide into organic combination (1, 8, 9). I^{131} accumulates rapidly in the gland after administration, but is discharged from the gland immediately upon the oral administration of KSCN. The thiocyanate ion displaces iodide but not organically bound iodine from the gland. The thyroids of a second group (9) bind accumulated iodide into mono- and di-iodotyrosine, but form only limited amounts of thyroxine. The accumulated labeled iodine is not released rapidly from the gland, and no labeled substance other than iodide and thyroxine is present in the blood. It was suggested that the defect in these latter patients was at the step in hormone synthesis where iodotyrosyl groups are coupled to form the finished thyroid hormones.

Studies with I^{131} have been made on other patients with congenital goiter. Hubble (2) studied 2 of 4 cretinous siblings with goiter. Both accumulated I^{131} rapidly and to an abnormally high level in the gland. By chromatographic methods, thyroxine was found in one of the glands. KSCN failed to discharge a significant amount of I^{131} from the thyroid of the other. Similar observations have been reported on 12 similar patients from 5 families by McGirr and Hutchison (3). Abnormally large amounts of protein-bound I^{131} were found in the sera of several subjects. Chromatographic analysis of the serum of 1 patient disclosed the presence of an iodinated substance which was not thyroxine. Although McGirr and Hutchison inferred that this might be di-iodotyrosine, the substance was not identified. An interesting feature of several of the patients of Hubble and of McGirr and Hutchison was the rapid release of the labeled iodine from the gland after the peak uptake was reached. In this respect, at least, these patients resembled the one reported here.

Although mono- and di-iodotyrosine occur normally in the thyroid, they do not occur normally in the blood, nor have they hitherto been convincingly demonstrated in the blood under any circumstance other than after heavy irradiation of the thyroid gland. Roche and associates (10) have suggested that the reason these substances do not appear in the blood is that they are deiodinated in the gland by a deshalogenase for which they are specific substrates.

The finding of mono- and di-iodotyrosine in the serum of this patient appears to be unique. Rall (11) has reported the presence of large amounts of "diiodotyrosine" in the blood of several patients with various diseases of the thyroid, but no convincing evidence for the identity of this substance was presented. Taurog *et al.* (12) have found that the concentrated alkali used by Rall causes considerable breakdown of thyroxine. Using the most refined methods available, Laidlaw (13), Taurog *et al.* (12, 14), Rosenberg (15), and Gross and Leblond (16) have been unable to detect the presence of appreciable amounts of mono- and di-iodotyrosine in blood. Robbins *et al.* (17, 18) found no free iodinated compounds other than tri-iodothyronine and thyroxine in the blood of a large number of patients who had received large quantities of I^{131}, although there was I^{131} at the origin of their chromatograms. This proved to be thyroglobulin released by the radiation effect on the thyroid cells. From this spot various iodinated compounds have been recovered after elution and hydrolysis (19). Recently Benua and Dobyns (20) have reported the finding of small amounts of mono- and di-iodotyrosine in the blood of certain patients with Graves' disease and with cancer of the thyroid after the administration of therapeutic doses of I^{131}. Radiation effect was not thought to play a role in the appearance of mono-iodotyrosine. Identification, however, was in a single solvent system, and the possibility that the spots were metabolic products of thyroxine and tri-iodothyronine was not excluded.

The criticism might be raised that the mono- and di-iodotyrosine observed in our patient was an artefact of irradiation of the gland. This could not be true for several reasons. The actual amount of radiation delivered per gram of gland was small because of the large size of the gland. Also, mono- and di-iodotyrosine were observed in the blood within two hours of administration of the I^{131}. Finally, when labeled mono- and di-iodotyrosine appear in the blood after heavy radiation, they are found in thyroglobulin, which does not move from the origin in the solvent system employed here (19).

The early appearance and large proportion of tri-iodothyronine relative to thyroxine was an unexpected finding. In 6 patients representing several types of thyroid disease Dingledine *et al.* (21) found the maximal ratio of labeled tri-iodothyronine to thyroxine to be 0.19. As may be seen from Figure 3, the ratio was much higher in this patient.

When di-iodotyrosine is introduced into the blood it is rapidly degraded. In the rat the blood concentration falls to 10 per cent of its initial value within half an hour (22). In myxedematous man Albert and Keating (23) found the concentration to be 1 per cent of its initial value in eight hours. Injected di-iodotyrosine is excreted partly intact, partly as iodide, and

partly as degradation products. It is only partially precipitable with the serum proteins. Little seems to be known about the peripheral metabolism of mono-iodotyrosine.

The kinetics of Figure 2 can be speculatively interpreted as follows: mono- and di-iodotyrosine were rapidly formed and secreted by the hyperplastic thyroid gland. The inflection of the serum curve at seventy minutes marked the beginning of secretion of significant amounts of these substances. The early precipitous fall of the *in vivo* curve resulted from thyroidal release of mono- and di-iodotyrosine, and subsequent renal excretion of these or of iodinated products derived from these substances, which were maintained in high blood concentration by the fast release rate from the gland. This phase of the metabolism of the I^{131} was largely completed by forty-eight hours. The subsequent slower release rate from the gland represented the metabolic activity in the thyroid devoted to the formation, storage and release of minute amounts of thyroxine.

The defect in the metabolism of iodine illustrated by the patient described here differs from those of two groups of patients previously described (1, 9). There was no difficulty in the accumulation and binding of iodide. Rather, the difficulty appeared to be the excessive formation and release of hormone precursors. The similarities between the retention and release of I^{131} by this patient and those of Hubble (2) and McGirr and Hutchison (3) suggest that among non-endemic goitrous cretins, the defect displayed by our patient might be relatively common.

SUMMARY

1. A case of severe hypothyroidism with congenital goiter is reported.

2. Labeled mono- and di-iodotyrosine appeared in the blood within two hours after administration of I^{131}, increased in concentration, and then decreased until they were not detectable at seventy-two hours. Labeled tri-iodothyronine and thyroxine were also present in the serum.

3. The hyperplastic goiter and the hypothyroid state are regarded as consequences of a continuous and abnormal loss of hormone precursors from the gland.

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THYROID FUNCTION FOLLOWING HYPOPHYSECTOMY IN MAN*†

M. C. LI, M.D.,‡ J. E. RALL, M.D., J. P. MacLEAN, M.D.,§
M. B. LIPSETT, M.D.,‖ B. S. RAY, M.D.
AND O. H. PEARSON, M.D.

The Division of Clinical Investigation, Sloan-Kettering Institute, Memorial Center and the Department of Surgery (Neurosurgery), New York Hospital-Cornell University Medical College, New York, N. Y.

A SEPARATE term, pituitary myxedema, has been used to describe the condition of hypothyroidism caused by pituitary insufficiency (1, 2). This name has been used, not only because the etiology is different from that of primary hypothyroidism, but because the clinical syndromes appear to differ. In most instances pituitary myxedema is the result of destructive lesions of the anterior lobe of the hypophysis, either from tumors or infarction. Usually it is complicated by loss of adrenocorticotropic and gonadotropic hormone secretions. From a ,unctional standpoint, it is often difficult to determine whether or not complete destruction of the pituitary has occurred. Furthermore, the rapidity of alteration of thyroid function following ablation of all functioning anterior pituitary tissue, and the sequential relationship to the development of clinical hypothyroidism have not been clarified. This report deals with the effect of surgical hypophysectomy in 35 cases of metastatic cancer, both on the clinical status of the patient and on the results of certain laboratory tests for thyroid function.

MATERIAL AND METHODS

All patients subjected to hypophysectomy had metastatic cancer, and many of them had received varied endocrine treatment prior to this study

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‡ Damon Runyon Research Fellow.

§ McGlaughlin Fellow.

‖ U. S. Public Health Service Fellow.

TABLE 1. SERUM PROTEIN-BOUND IODINE, THYROIDAL I^{131} UPTAKE AND SERUM CHOLESTEROL VALUES BEFORE AND AFTER HYPOPHYSECTOMY

Patient	Age (yrs.)	Sex	Carcinoma	Endocrine status	Serum PBI (μg./100 cc.)		Thyroidal uptake of I^{131} (%)		Serum cholesterol (mg./100 cc.)	
					Before	After*	Before	After*	Before	After†
1. L.W.	48	F	Breast	Adrenalectomized; oophorectomized	5.8	0.3	12.0	0	235	188
2. A.G.	42	F	Breast	Adrenalectomized; oophorectomized	4.0	1.5	30.0	5.6	194	272
3. M.C.	54	F	Breast	Postmenopausal; adrenalectomized	7.1	0.2	21.8	4.6	219	215
4. G.B.	42	F	Breast	Oophorectomized	3.2	0.8	27.0	3.2	100	208
5. I.M.	33	F	Breast	Oophorectomized	8.4	1.0	25.3	8.6	117	200
6. C.S.	37	F	Breast	Oophorectomized	5.9	2.0	27.0	6.3	—	—
7. A.W.	39	F	Breast	Oophorectomized	5.1	2.0	32.0	5.1	210	325
8. E.B.	60	F	Breast	Postmenopausal	5.2	0.2	38.0	7.4	170	318
9. R.F.	43	F	Breast	Normal	4.0	0.5	26.0	2.7	108	235
10. F.H.	50	F	Breast	Postmenopausal	2.8	0	38.0	7.4	133	272
11. W.M.	37	F	Breast	Oophorectomized; adrenalectomized	10.1	0.7	32.0	515	204	164
12. H.M.	70	F	Breast	Postmenopausal	4.0	1.3	28.8	2.5	187	412
13. M.Mc.	49	F	Melanoma	Normal	6.0	0	25.0	1.7	133	147
14. M.P.	33	M	Melanoma	Normal	4.2	2.8	32.4	3.2	176	214
15. P.Y.	32	F	Melanoma	Normal	7.1	1.6	21.3	4.3	—	—
16. M.T.	35	F	Melanoma	Normal	4.5	1.5	22.4	1.5	153	201
17. J.N.	65	M	Prostate	Orchiectomized	7.7	1.9	19.0	1.3	308	229
18. J.R.	70	M	Prostate	Orchiectomized	5.4	1.4	8.5	2.2	223	216
19. M.R.	37	F	Breast	Menopausal	4.3	0.9	15.6	7.3	226	300
20. R.D.	33	F	Breast	Oophorectomized; adrenalectomized	5.6	1.0	31.4	3.5	199	342
21. M.E.	50	F	Breast	Postmenopausal	3.6	1.3	29.0	3.2	—	—
22. V.E.	52	F	Breast	Oophorectomized; adrenalectomized	—	0.9	14.0	2.9	182	252
23. E.N.	54	F	Breast	Postmenopausal	2.8	1.6	16.0	0.7	186	176
24. N.C.	34	M	Embryonal	Orchiectomized	6.9	2.6	35.2	8.8	220	367
25. R.G.	52	F	Breast	Oophorectomized; adrenalectomized	—	—	23.0	5.6	179	290
26. M.D.	49	F	Breast	Postmenopausal	—	—	38.4	3.7	—	—
27. S.S.	67	M	Prostate	Orchiectomized	—	—	38.0	2.0	250	271
28. M.F.	50	F	Breast	Adrenalectomized; oophorectomized	4.2	—	25.7	8.8	—	—
				Mean	5.75	1.2	28.3	4.6	189.2	250

* The values after hypophysectomy are the lowest noted for each patient.
† The values after hypophysectomy are the highest noted for each patient.

(Table 1). Hypophysectomy was performed by a transfrontal approach in most of the cases (3). The patients received cortisone in large doses twelve hours before, and for a few days after surgery. The dosage was almost identical to that used in patients undergoing adrenalectomy (4). After hypophysectomy, all patients were maintained on oral cortisone (average dose, 50 mg. per day). Some patients also required Pitressin for control of water balance. With this regimen, the general condition of the patients was well maintained. The nutritional status and the level of serum total protein were normal in these patients, some of whom gained weight

and showed a positive nitrogen balance following hypophysectomy.

Serum concentrations of protein-bound iodine and total iodine were determined by the method of Barker, with the modification suggested by Riggs (5, 6). In addition, recovery of iodine in all analyses was checked with I^{131}, and appropriate correction was made for losses during the analyses.

Radioiodine was given in tracer amounts and the urinary excretion and 48-hour thyroidal uptake were measured.

Urinary gonadotropins were assayed by the method of Klinefelter, Albright and Griswold (7).

Completeness of hypophysectomy was evaluated on the basis of 1) disappearance of urinary gonadotropin, and 2) occurrence of adrenal crisis upon withdrawal of cortisone.

Completeness of hypophysectomy was also confirmed by postmortem examination when possible. On the basis of these criteria, 28 of the 35 patients were considered to have had complete hypophysectomies, and 7, partial hypophysectomies. The changes in thyroid function which are presented in the following study were well correlated with the foregoing criteria.

RESULTS

1. *Effect of hypophysectomy on the uptake of radioiodine by the thyroid.* Figure 1 is a scattergram showing the uptake of radioiodine before and after surgery in 28 patients who had total hypophysectomy. The values plotted after hypophysectomy are the first to fall within the myxedema range. Except for 3 patients with low values, the prehypophysectomy results are within the normal range. The mean value for the 48-hour radioiodine uptake was 28.3 per cent, which compares well with the normal values from this laboratory and others (8, 9). The radioiodine uptake after hypophysectomy in those subjects in which the operation had been complete was usually at a low level within four weeks.

The rate of decline in I^{131} by the thyroid following hypophysectomy was measured in Patient M. Mc. (Fig. 2). Although adequate data are not available to determine early changes in radioiodine uptake, in most of the patients it appeared that I^{131} uptake had fallen maximally within two to four weeks after hypophysectomy. In 2 patients who appeared to have complete hypophysectomies by other criteria, the thyroidal radioiodine uptake did not fall to below 8 per cent until three months after hypophysectomy. The mean value of the lowest I^{131} uptake following total hypophysectomy in these 28 patients was 4.6 per cent (Table 1).

Of the 7 patients who had incomplete hypophysectomy, 5 showed no significant changes in radioiodine uptake over a period of several months,

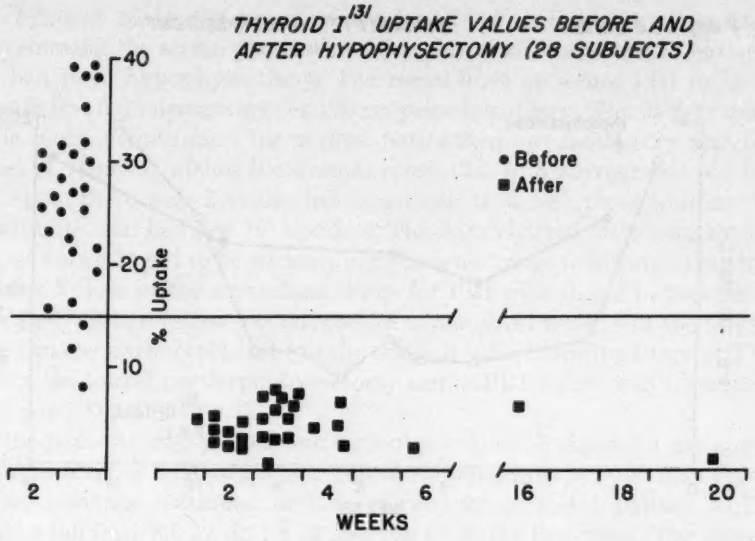

FIGURE 1

and 2 patients showed transient decreases. Figure 3 illustrates the transient suppression of thyroid function in Patient G.P., who had an incomplete hypophysectomy.

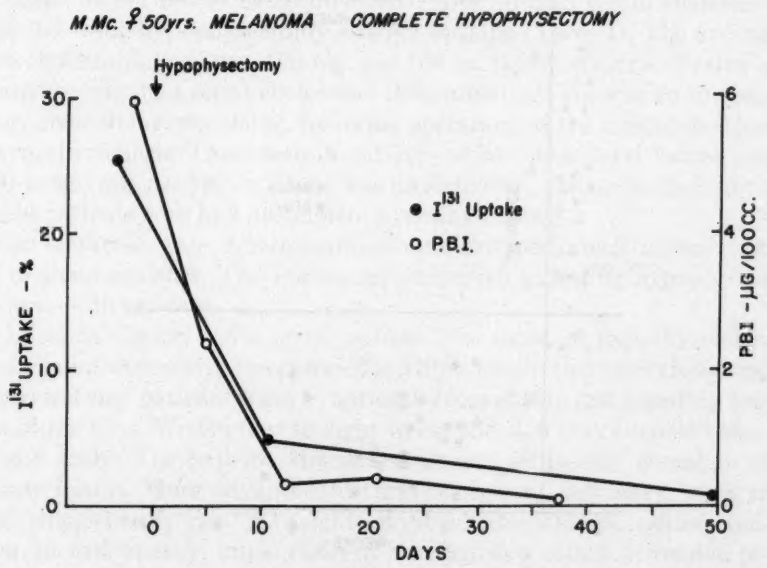

FIGURE 2

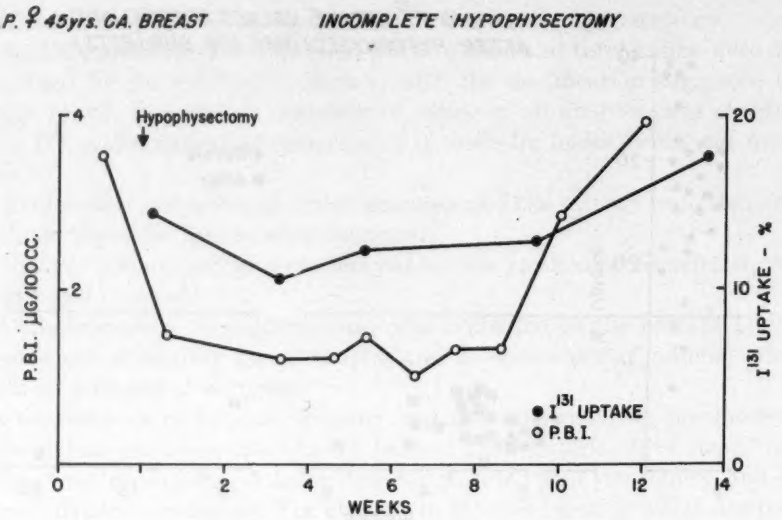

FIGURE 3

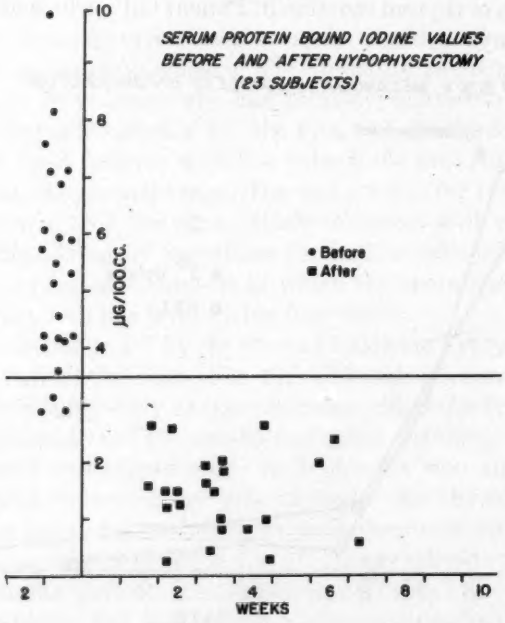

FIGURE 4

2. *Effect on the level of serum protein-bound iodine.* Figure 4 is a scattergram showing the serum protein-bound iodine (PBI) values in 23 patients who had total hypophysectomy. The mean level of serum PBI in these patients was 5.8 micrograms per 100 cc. prior to surgery. This is very close to the mean value found for normal patients in our laboratory and the spread of values is within the normal range (3.5 to 8 micrograms per 100 cc.). Again there were 3 values below normal. However, these were not in the patients who had low I^{131} uptakes. The data charted after surgery are the first values found to be within the myxedema range following hypophysectomy. Values in the myxedema range for PBI were found in 2 patients in the first week, 6 in the second week, 8 in the third week, 4 in the fourth week, 2 in the sixth week, and 1 in the seventh week following surgery. The mean of the lowest posthypophysectomy serum PBI values was 1.2 micrograms per 100 cc. (Table 1).

In the patients with incomplete hypophysectomy, 3 showed a sustained fall in the level of PBI to a value below 3 micrograms per 100 cc. (Table 2). Two patients continued to have normal levels and 1 patient, G.P., showed a fall from 3.6 μg. to 1.4 μg. per 100 cc. in the first week. The values then returned to 2.8 μg. and 3.9 μg. per 100 cc. at the end of the tenth and twelfth weeks (Fig. 3). The fall and subsequent rise of serum PBI concentration were accompanied by similar changes in thyroidal radiodine uptake.

3. *Effect on the level of serum cholesterol.* Alteration in serum cholesterol values following hypophysectomy was not striking (Table 1). The average serum cholesterol level was 190 mg. per 100 cc. before surgery. Twelve of 23 patients who had serial cholesterol determinations showed an increase of more than 50 mg. per 100 cc. following operation, at the time of developing hypothyroidism. There were 5 patients whose cholesterol values rose to 300 to 420 mg. per 100 cc. There was no significant change in cholesterol levels in patients who had incomplete hypophysectomy.

Basal metabolic rate determinations were not performed routinely in most of these patients. The lowest determination following hypophysectomy was −36 per cent.

4. *Effect on clinical status of the patient.* The onset of hypothyroidism is gradual and varies with the individual. Often within two weeks following hypophysectomy patients begin to notice dryness of skin and a grating sensation of the eyes. Within four to eight weeks the skin may become thickened and scaly. The patients stop sweating and frequently complain of cold extremities. More advanced features of thyroid deficiency, such as mental sluggishness, gain in weight, myxedematous facies, sallow complexion, mental apathy, impairment of hearing, slow return of tendon reflexes and somnolence were also observed. Eight patients showed electro-

TABLE 2. SERUM PROTEIN-BOUND IODINE AND THYROIDAL I^{131} UPTAKE VALUES BEFORE AND AFTER INCOMPLETE HYPOPHYSECTOMY

Patient	Age (ys.)	Sex	Carcinoma	Endocrine status	Serum PBI (μg./100 cc.) Before	Serum PBI (μg./100 cc.) After	Thyroidal uptake of I^{131} (%) Before	Thyroidal uptake of I^{131} (%) After
1. S.H.	43	F	Ovary	Normal	6.7	3.5 1.3	12.6	1.1 6.3
2. M.DoW.	48	F	Melanoma	Normal	6.8	2.7	35.0	26.0 34.6
3. J.V.	23	F	Chorio-epithelioma	Normal	—	—	—	38.0
4. G.P.	46	F	Breast	Oophorectomized	3.5	1.0 3.9	—	11.0 17.0
5. M.D.	55	F	Breast	Postmenopausal	—	6.8	—	18.0 5.2 38.0
6. L.B.	37	F	Breast	Oophorectomized	6.1	6.0	—	31.0 21.0 37.0
7. B.S.	49	F	Breast	Postmenopausal	—	1.3	—	26.0 27.0

cardiographic changes, such as lowering of voltage, decreased amplitude of T waves and sinus brachycardia. There were only 2 patients who showed slight enlargement of the heart. The time required for the development of obvious hypothyroidism varied, the earliest period being four weeks and the latest sixteen weeks after hypophysectomy; the average period was eight weeks. Two exceptions were noted. These were in women who did not show any of the foregoing features eight and twelve months, respectively, after hypophysectomy despite low thyroidal radioactive iodine uptake and a low level of serum PBI.

The clinical management of hypothyroidism was not difficult. Desiccated thyroid (120 mg. daily) or triiodothyronine (50 to 75 μg. daily) caused myxedematous patients to revert to a euthyroid status in three to six weeks and in one to two weeks, respectively, as noted in the treatment of primary myxedema. It was not necessary to begin treatment gradually.

5. *Effect on response of the thyroid gland to TSH.* Figure 5 illustrates the rapid response to stimulation of the thyroid gland by a single intramuscular injection of 40 units of TSH[1] given eight months after hypophysectomy to a patient who had had no thyroid hormone replacement therapy. The level of serum PBI rose from 2.5 μg. to 5.6 μg per 100 cc. in twelve hours; it reached a peak of 8 μg. per 100 cc. in forty-eight hours, but returned to

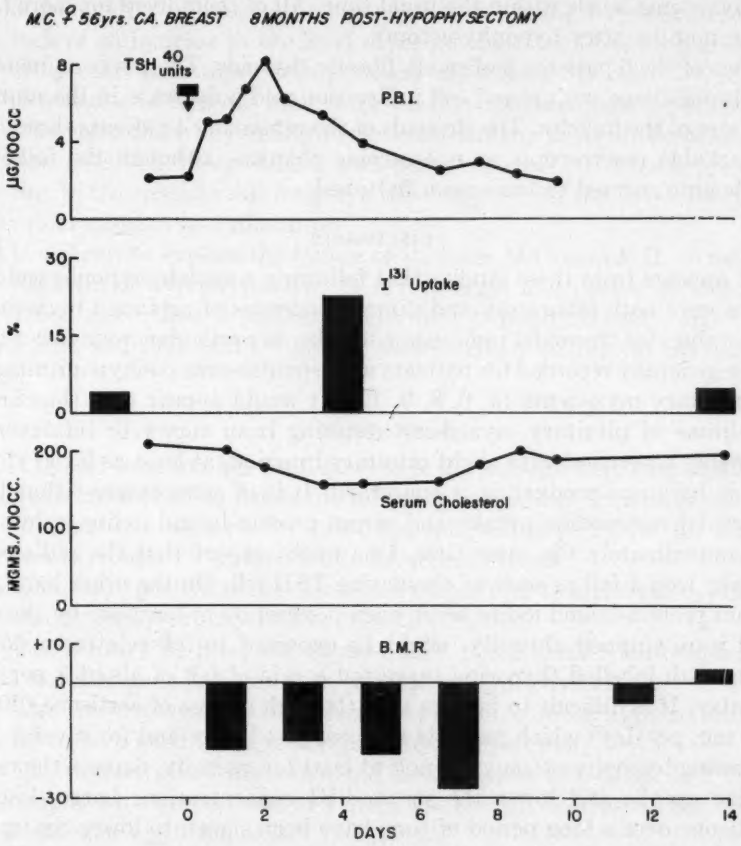

FIGURE 5

the myxedema level in six days. Accompanying this, there was a rise of thyroidal I^{131} uptake from 4 per cent to 20 per cent; later the uptake fell to 4 per cent. There was no significant alteration in serum cholesterol

[1] Thytropar, obtained through the courtesy of the Armour Laboratories, Chicago, Ill.

values during this experimental period. The basal metabolism rose to −4 per cent eleven days after the administration of TSH.

6. *Effect on histologic appearance of the thyroid.* Specimens of the thyroid glands of 6 patients were available from postmortem examinations. All of the 6 patients had had definite hypothyroidism and were treated for this condition before death. Their serum protein-bound iodine levels and thyroidal I^{131} uptakes were normal before hypophysectomy and fell to myxedema levels within the usual time. All of them lived for more than three months after hypophysectomy.

Two of the 6 patients had small, fibrotic thyroids. There was an increase of fibrous tissue with round-cell infiltration and a decrease in the number and size of the follicles. The thyroids of the remaining 4 patients showed no remarkable macroscopic or microscopic changes, although the follicular epithelium seemed to have been flattened.

DISCUSSION

It appears from these studies that following complete hypophysectomy there were both laboratory and clinical evidences of profound myxedema. The values for thyroidal radioiodine uptake, in particular, were lower than those generally recorded for patients with spontaneous panhypopituitarism or pituitary myxedema (5, 6, 8, 9, 10). It would appear that the clinical syndrome of pituitary myxedema resulting from tumor or infarction is probably associated with slight pituitary function, at least as far as thyrotropic hormone production is concerned. It is of some interest that both thyroidal radioiodine uptake and serum protein-bound iodine values fell at approximately the same time. One might expect that the radioiodine uptake would fall as soon as circulating TSH fell. On the other hand, the serum protein-bound iodine level, even if secretion of hormone by the thyroid were stopped abruptly, might be expected to fall relatively slowly. Data with labelled thyroxine suggested a rate of fall of about 8 per cent per day. It is difficult to be sure that the high dosage of cortisone (200 to 300 mg. per day) which patients received just before and for several days following hypophysectomy did not, at least temporarily, depress the radioiodine uptake and lower the serum PBI concentration. Large doses of cortisone over a long period of time have been shown to lower the uptake of radioiodine and occasionally to depress the serum PBI level (11). Hence, interpretation of relative rates of fall of thyroidal I^{131} uptake and serum PBI level is not clear.

No data are available on the precise effect of hypophysectomy on thyroidal function in terms of the relative effect on the iodine trap or the rate of iodine binding. However, in view of the marked fall in uptake, it seems likely that the iodine trap was considerably diminished. One result which

is clear from the data presented, is the reliability of thyroidal radioiodine uptake and serum PBI determinations in the evaluation of the completeness of hypophysectomy. This is particularly well demonstrated in the patients who had a transient fall in hypophyseal function.

It is not clear why the serum cholesterol values did not show a more uniform rise in patients with complete hypophysectomy. This did not appear to be related to the fact that the patients were debilitated and their food intake frequently diminished in the months following hypophysectomy. The lack of an increase in the level of serum cholesterol has been also observed by other investigators in pituitary myxedema (12, 13).

The features of hypothyroidism in the hypophysectomized patient do not differ from those of primary myxedema. Enlargement of the heart has not been observed, in agreement with other reports (14). This is presumably due to the considerable length of time required for the development of pericardial effusion and dilatation.

It is difficult to explain the failure of Patients M.C. and F.H. to exhibit any stigmata of hypothyroidism. It is now eight and twelve months, respectively, since these 2 patients underwent hypophysectomy, and their serum PBI and thyroidal I^{131} uptake values remain within the myxedema range.

SUMMARY

1. Thyroid function has been studied in 28 patients following complete hypophysectomy and in 7 patients following incomplete hypophysectomy.

2. Within four weeks after total hypophysectomy, values for serum PBI and thyroidal I^{131} uptake fell to myxedema levels.

3. Clinical hypothyroidism developed within four to eight weeks following total hypophysectomy in 26 of the 28 patients.

4. The serum PBI level and thyroidal I^{131} uptake either fell transiently or did not change in patients with incomplete hypophysectomy.

5. The development of both laboratory and clinical hypothyroidism appears to be a good index of complete hypophysectomy.

6. The thyroid gland was examined histologically in 6 cases. In 2 there was atrophy with fibrosis, and in 4 there was a slight decrease in the height of the follicular epithelial cells.

Acknowledgment

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RESULTS OF SURGICAL HYPOPHYSECTOMY IN A CASE OF MALIGNANT EDEMATOUS EXOPHTHALMOS

M. ALBEAUX-FERNET, J. GUIOT, S. BRAUN AND J. D. ROMANI

Centre d'Endocrinologie, Hôpital Laënnec and Hôpital Foch, Paris, France

WE BELIEVE it of interest to report a case of malignant exophthalmos aggravated by treatment of the associated hyperthyroidism and apparently cured by hypophysectomy. Evidence was obtained by histochemical studies of the pituitary, of hypersecretion of thyroid-stimulating hormone (TSH).

CASE REPORT

Mrs. L.H., aged 56, proprietress of a café, consulted us on January 11, 1954. She had several complaints, including palpitation and precordial pain. She had lost 7 Kg. in weight during the preceding few months, without any intestinal disturbance, and her family had noted increasing nervousness.

A painstaking history revealed a normal, although overactive psyche. No single important psychiatric trauma was noted, but rather a series of minor ones. The menopause had occurred uneventfully three years before.

We were at once struck by the intensity of the patient's stare and the degree of protrusion of the eyeballs, even though the latter were buried in palpebral edema, which was most marked in the upper lids. Further examination showed the degree of exophthalmos to be 18 mm. in both eyes (Hertel exophthalmometer set at 118 mm.). The signs of Stellwag, von Graefe and Moebius were present, the visual fields were normal, the visual acuity was 6/10, the ocular tension was slightly increased, and the fundi were intact. The pulse rate was 130, the extended fingers showed a slight tremor, and the thyroid gland was just palpable.

The heart sounds were normal and regular but forceful. The electrocardiogram showed sinus tachycardia. The BMR was +130 per cent. In other respects the physical examination was not remarkable.

The patient was considered to have hyperthyroidism, in spite of the absence of marked enlargement and vascularity (bruit) of the thyroid gland, and of generalized tremor.

The ocular phenomena were thought to indicate the presence of hyperpituitarism as well as hyperthyroidism. A rapid cure of the hyperthyroidism was considered inadvisable for this reason.

The events which followed may be grouped into three periods.

Period I (January 11–April 29, 1954)

The treatment decided upon was complete rest with sedation, vitamin A, and *n*-butyl 3-5-diiodo-4-hydroxybenzoate (Diobene) in a dosage of 150 mg. per day. On February 11 the patient appeared more calm and no longer complained of palpitation and pre-

cordial discomfort. The pulse was 90, the weight was stationary. Measurements of the exophthalmos remained unchanged but the eyes were suffused and the patient noted transitory sensations of a mist passing before them. From February 11 to March 19 Diobene was omitted and testosterone (50 mg.) and estrogen (Dienestrol, 25 mg.) were injected in microcrystalline suspension.

Despite continued improvement in the general condition the exophthalmometer reading increased slightly (Fig. 1) and there was an increase in the congestive phenomena, especially in the right eye, which showed a blepharoconjunctivitis with tearing and injection of sclera. The globes were harder on palpation. Ophthalmologic examina-

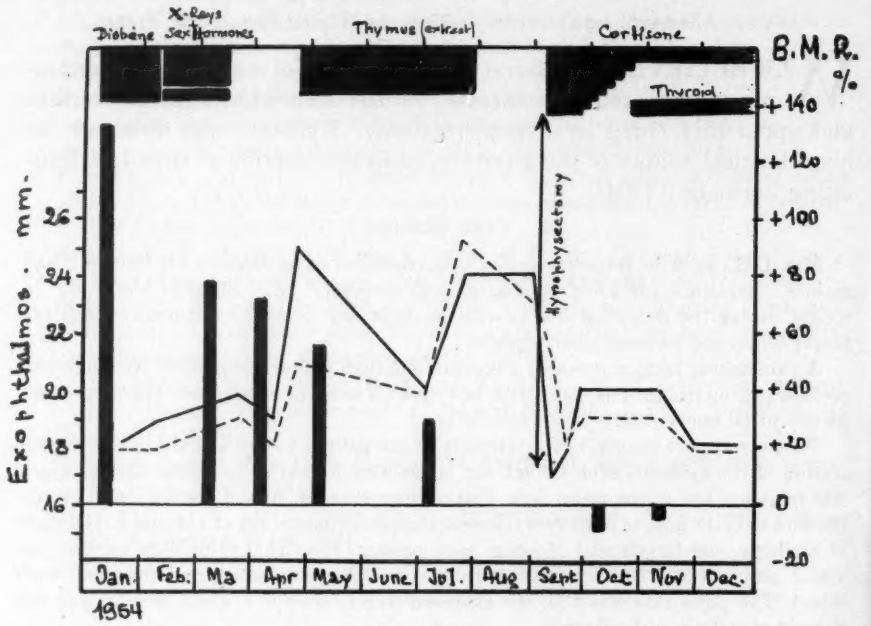

FIG. 1. Course of the exophthalmos and BMR during various types of therapy, before and after hypophysectomy. (The straight lines denote the exophthalmos in the right eyeball; the broken lines denote the exophthalmos in the left eyeball. The solid black columns denote the BMR.)

tion showed no change in visual acuity (6/10 on both sides); fundi and visual fields were normal. The ocular tension was 32 in the right eye and 28 in the left.

An attempt was then made to inhibit the overactive pituitary with x-ray therapy.

During the month of March a total of 900 r. was administered to the pituitary through six portals.

From March 20 to April 12, the patient was hospitalized. During the hospitalization there was a weight gain of 2 Kg.

The resting pulse was 90. The exophthalmos was little changed but was slightly greater on the right (19 mm. right; 18 left). Under local treatment with vasoconstrictors, there was some decrease in the congestive phenomena and the ocular tension fell (28

right and 28 left). The BMR fell to +75 per cent. The patient's general condition was satisfactory on leaving the hospital on April 12.

Period II (April 29–September 3, 1954)

On April 29 the patient returned with considerable exacerbation of her exophthalmos (25 mm. right; 21 mm. left) and a marked blepharo-conjunctivitis. The BMR was +54 per cent. On May 28 she was hospitalized for a trial of treatment with extract of thymus, based on the experimental neutralization of TSH by thymus extracts (1).

For forty days the patient received parenterally the equivalent of 20 Gm. of fresh thymus gland. At the end of this time the exophthalmos had receded (20.5 mm. right; 20 mm. left) and had lost its inflammatory character. The BMR had fallen to +35 per cent.

Immediately following her discharge on July 5 the exophthalmos began to progress despite continuation of treatment, reaching 24 mm. right and 25 mm. left. It was accompanied for the first time by retro-orbital pain. On July 26 the patient was admitted to the ophthalmologic service with keratitis and iridocyclitis of the right eye. The ocular tension was 77 right and 40 left.

A blepharorraphy was performed and local treatment with pilocarpine, Neo-synephrine and hydrocortisone instituted.

Treatment with thymic extract was discontinued. At the end of a month the cornea had healed and ocular tension had decreased, but the exophthalmos remained practically unchanged. Examination of the fundi showed flat pale disks, indicating beginning optic atrophy. There was a relative central scotoma on the left and an absolute central scotoma on the right. The vision in the left eye was reduced to 1/20. Exophthalmometric measurements were 24 mm. right and 23 mm. left.

Period III (September 3–September 13, 1954)

The clinical picture was that of malignant exophthalmos in a patient with decreasing hyperthyroidism. Both local and general manifestations attested to the gravity of the situation.

Local signs. We have learned to associate certain local signs with malignant edematous exophthalmos, as distinguished from the usual exophthalmos associated with thyrotoxicosis. In this patient they were: marked palpebral edema, marked congestive and inflammatory signs, irreducibility of exophthalmos, intraocular hypertension, and optic atrophy with loss of vision.

General signs were: continued fever (38°C.) without evidence of infection, weight loss, torpor, muscular weakness and throbbing headache. These symptoms, suggestive of thyroid storm, had appeared before death in the previous 2 patients with malignant exophthalmos observed by us.

The foregoing considerations induced one of us (MAF) to take the responsibility for advising surgical hypophysectomy. The alternatives—cortisone therapy and the Naffziger decompression operation—were rejected as being palliative only.

Hypophysectomy was performed on September 13, 1954, by Dr. Guiot at the Foch Hospital. The operative note mentions enlargement of the ventricles. They contained 40 cc. of fluid, which showed no increase in protein or cellular elements.

The pituitary gland appeared slightly larger than normal. It was removed as completely as possible by curettage.

Although the postoperative course was uneventful on the whole, on the second night after the operation there was sudden loss of consciousness, general deterioration, left-

sided weakness, and spastic movements (including chewing motions) on the right side. These signs were interpreted as indicative of hypoglycemia and appeared to respond to intravenous glucose, although the blood sugar level in the emergency specimen drawn before treatment was reported later as 80 mg. per 100 cc. Cortisone in a dosage of 150 mg. daily was added to the high carbohydrate intake. Thereafter improvement was rapid. On September 16 the patient, her eyes still bandaged, recognized us by our voices. On September 18 she got out of bed and fed herself. She remained apathetic, however, and spoke in a monotonous voice. September 19 a distinct decrease in palpebral edema was noted. The eyes were less congested and the exophthalmos substantially reduced (Fig. 1).

The ophthalmologic findings, which showed progressive improvement during the two months following the operation, are shown in Table 1. The visual acuity became nearly

TABLE 1. THE EFFECT OF HYPOPHYSECTOMY ON OPHTHALMOLOGIC FINDINGS

	Exophthalmos (mm.)		Visual acuity		Ocular tension	
	Rt.	Lft.	Rt.	Lft.	Rt.	Lft.
Preoperative	24	23	0	1/10	77	40
	Hypophysectomy Sept. 13, 1954					
Sept. 20	17	21	—	—	25	27
Sept. 29	18	18	2/10	3/10	35	35
Oct. 16	20	19	4/10	4/10	35	25
Oct. 25	20	19	5/10	4/10	32	28
Nov. 4	20	18	7/10	8/10	—	—
Nov. 17	20	19	—	—	—	—
Nov. 29	18	18	—	—	—	—
1955						
Jan. 12	21	20	8/10	2/10		

normal at the end of the first month. There has been no further change in the residual exophthalmos (18 right; 18 left.)

On September 27 the patient returned to the Laënnec Hospital. Her general condition had begun to improve; she became more alert, her appetite returned, and her weight increased steadily from 47 to 54 Kg.

There was at first a definite diabetes insipidus (urinary output <6 liters per twenty-four hours). This responded to small doses of posterior pituitary extract and began to subside spontaneously by the seventh week.

The measurements of endocrine status before and after operation are given in Table 2. The interpretation of thyroid and adrenal function is complicated by the substitution therapy which was required.

At the time of writing (four months after hypophysectomy) it is not yet certain how much pituitary function will return. Although the scalp hair has regrown to nearly normal length, the axillary hair (shaved in

December) has not regrown perceptibly and arm and leg hair are still absent. The pubic hair is sparse.

The face has the soft fine skin and pallor associated with clinical pituitary insufficiency. The urinary output on an unrestricted fluid intake ranges from 1,500 cc. to 2 liters daily.

Specimens of the pituitary tissue removed were fixed in Bouin-Hollande solution without acetic acid. Some sections were stained by the classic techniques (hematoxylin-eosin and Mallory's trichrome). Others were

TABLE 2. LABORATORY FINDINGS BEFORE HYPOPHYSECTOMY (SEPTEMBER 13, 1954), AND TWO AND FOUR MONTHS AFTERWARD

	Before hypophysectomy (Apr. 1954)	Two months after hypophysectomy (Nov. 1954)	Four months after hypophysectomy (Jan. 1955)
Treatment	—	Cortisone, 20 mg./day	Desicc. thyroid
BMR	+54%	+2%	−20%
Serum PBI (μg./100 cc.)	8.25	7.0	—
Serum cholesterol (mg./100 cc.)	250.0	212.0	214.0
Serum phosphorus (mg./100 cc.)	3.9	3.2	—
Urinary 17-ketosteroids (mg./24 hr.)	9.3	8.5	2.1
Urinary 17-hydroxysteroids (mg./24 hrs.)	—	4.8	4.6
Urinary FSH (m.u./24 hrs.)	192	< 5.8	—
Thorn test (ACTH)	positive eos. (−98%) 17-KS (+130%)	negative eos. (+79%) 17-KS (−20%)	—

stained to show glycoproteins by the PAS method of MacManus (2, 3), consisting of exposure to amylase followed by counter staining with hematoxylin and orange G. The pathologic changes observed involved: 1) the architecture of the gland, 2) its cellular composition, and 3) the cytologic characteristics of the cells.

Under low magnification the trabecular structure characteristic of the normal pituitary was seen to be replaced by an alveolar arrangement of the cells similar to that seen in pregnancy, but differing from it in the greater uniformity of the cells contained in the alveoli and the relative paucity

of colloid (Fig. 2). The tissue appeared strikingly uniform. Islands of acidophilic cells (stained red by eosin and yellow by orange G) stood out clearly against this uniform ground. These cells were pale and poorly granulated but not otherwise unusual. The great majority of the cells were large, clear, and without granules when stained by hematoxylin and eosin; their nuclei were large and poor in chromatin. These cells were round or somewhat polygonal, but the cytoplasm was not sharply outlined.

The PAS technique, specific for polysaccharides showed many fine ir-

FIG. 2. The anterior pituitary gland shows an alveolar structure. In the right half of the preparation, large clear cells are predominant; their outlines are vague. These are the so-called basophil alpha cells. (hemalum-phloxine-saffron; low-power)

regularly distributed granulations less the 1 μ in diameter (Fig. 3). In some cells these granules coalesced to form a fine network staining brightly with fuchsin and bisulfite. In addition, in some cells there were larger droplets of glycoprotein in vacuoles.

These cellular elements correspond to the "sparsely granulated basophile cells" of Mellgren, called by Pearse (4) "intermediate mucoid cells" or "amphophile cells." They constitute, in man, the equivalent of the basophil cells in the pituitary of the rodent.

It appears possible to follow a secretory cycle in these cells. In some of

them the granules are more sparse at the periphery and in others have disappeared. This is associated with an increased volume of these cells and an hypertrophy of the nucleus with a tendency, exceptionally, to be bilobed. The presence of these hypertrophied amphophils is thought to indicate an intense secretory activity of the gland. In contrast to the considerable hyperplasia of the amphophils the rarity of basophil cells was striking. These last are easily recognized by their pronounced basophilia and other

FIG. 3. High-power view of anterior pituitary. In the cytoplasm of the thyreotrope cells, there are tiny glyco-protein granules which are sometimes disposed in a network formation, but at other times appear as vacuoles. These characteristics differentiate them from the acidophil cells. The latter are smaller and rounder; they also have well defined borders and denser cytoplasm. (PAS by Purves and Griesbach's technic)

cytologic characteristics, such as clearly outlined cytoplasm, round or oval shape, great density of PAS positive granules and the punched-out appearance of the vacuoles. The chromophobe cells were grouped in rare islands and were not remarkable in appearance.

Certain collagen-containing areas of sclerosis were observed in which the cells appeared involuted and showed little sign of secretory activity. These were thought possibly to be a result of x-ray therapy.

In general, sections of the pituitary removed at operation showed a

marked deviation from the normal structure. There was evidence of an increase in amphophil cells in various stages of secretory activity. These cells are thought to be the site of production of thyrotropic hormone. The changes described are consistent with those reported in a cretin by Burt and Cohen (5) and in a case of Herlant's (6).

SUMMARY

A case of rapidly progressive malignant exophthalmos is presented, in which marked amelioration of local and general symptoms followed surgical hypophysectomy.

Histologic examination of the pituitary tissue removed at operation showed an increase in the numbers and in the secretory activity of cells which are considered to be the site of TSH production.

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ACUTE EFFECTS OF INTRAVENOUS HYDROCORTISONE ON GLUCOSE AND INSULIN TOLERANCES, AND LEVELS OF SERUM AND URINARY INORGANIC PHOSPHORUS

MURRY PERSKY, M.D., JOSEPH LINSK, M.D., MARIAN ISAACS, M.D., JOHN P. JENKINS, M.D., MILTON ROSENBLUTH, M.D. AND HERBERT S. KUPPERMAN, PH.D., M.D.

The Department of Therapeutics, New York University-Bellevue Medical Center, and Third (New York University) Medical Division, Goldwater Memorial Hospital, New York, N. Y.

INTRODUCTION

ADRENOCORTICAL hormones directly and adrenocorticotropic hormone (ACTH) indirectly will induce hyperglycemia, glycosuria and diabetic-like glucose tolerance curves in man and animals (1, 2, 3). However, the extent of the decrease in glucose tolerance varies, on an average dose of 100 mg. of cortisone a day, from only occasional impairment (4) to profound glycosuria observed on the third day of treatment, and a marked diabetic curve on the sixteenth day (5). We have previously demonstrated significant fasting hyperglycemia and reduction of glucose tolerance following intravenous infusion of ACTH (6, 7). There was, however, no linear relationship between increasing doses of ACTH and the extent of the changes in glucose tolerance. It was shown by Forsham *et al.* in 1948 that the apparent diabetogenic effect of ACTH is mediated through the adrenal cortex (8).

An anti-insulin effect of adrenocortical steroids and ACTH has been demonstrated in mice in 1940 and in man in 1953 (6, 7, 9). Colowick *et al.* showed in 1947 that adrenocortical extracts will inhibit the hexokinase reaction, but that insulin can, in turn, abolish this effect (10). In 1951, Conn and associates reported that insulin resistance could be demonstrated after only three days of treatment with 100 mg. of cortisone a day (11). Opposing this were the data of Zimmerman *et al.* in 1953, which failed to demonstrate any anti-insulin effect of ACTH in man (12).

The most commonly accepted mechanisms of action of the 11-oxysteroids on carbohydrate metabolism are: a) increased gluconeogenesis (13),

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b) increased deposition of glycogen in muscle and liver (8, 14–16), and c) decreased peripheral utilization of glucose (2, 3, 17). The net effect of these actions is a decrease in glucose tolerance.

The findings in this paper bear mainly on the third mechanism, namely, the effect of intravenous compound F on peripheral glucose utilization. Evidence against the concept of decreased peripheral utilization is presented by the experiments of MacBryde and de la Balze, who demonstrated an increased peripheral glucose utilization in patients with Addison's disease being treated with cortical extracts rich in 11-oxysteroids (18). Also Ingle *et al.* in 1947 could not demonstrate inhibition of peripheral glucose uptake over a four-hour period with adrenocortical extracts (19).

In general, the mechanisms in peripheral glucose metabolism in humans have been inferred from the findings in animal perfusion experiments (20) and experiments in viscerectomized preparations (19). However, Forsham and Thorn have presented and confirmed the evidence that the fall in the level of serum inorganic phosphorus during glucose administration has become a valid criterion for the peripheral utilization of glucose (20). Changes in serum phosphorus concentration have been reported by Ingbar *et al.* with the use of compound E and ACTH in experiments unrelated to glucose metabolism. The hormonal substances greatly increased urinary phosphorus excretion with constant rates of filtration, suggesting either a reduced tubular reabsorption or an increased tubular secretion of phosphate, or both (21). In 1953 Roberts and Pitts confirmed this observation and indicated that the renal tubular reabsorption capacity for phosphate is decreased with compound E but not with desoxycorticosterone acetate (22).

The purpose of the present investigation was to demonstrate the effects of intravenous hydrocortisone (compound F) infused over a four-hour period on glucose tolerance, insulin tolerance and the levels of serum and urinary inorganic phosphorus.

METHODS

Standard 100-Gm. single-dose oral glucose tolerance tests were performed on 8 nondiabetic patients and 5 patients with decreased glucose tolerance maintained on an adequate carbohydrate intake. After a period averaging one week, the same patients received infusions containing 100 mg. of 17-hydroxycorticosterone (hydrocortisone) in 20 cc. of 50 per cent alcohol and diluted in 500 cc. of physiologic saline. The preparation was given intravenously over a four-hour period. Venous blood glucose levels were determined serially by a modified Smogyi-Schaeffer-Benedict method. This procedure was designated as the *hydrocortisone tolerance test.*

Similar infusions were given to the 8 patients with normal glucose toler-

ance and to the patients with decreased glucose tolerance after another week, except that oral glucose tolerance tests were performed following the administration of 100 Gm. of glucose simultaneously with the onset of the hydrocortisone infusion. Blood glucose levels were again determined serially. This was designated as the *hydrocortisone-glucose tolerance test.*

Serum inorganic phosphorus levels were obtained in 5 patients with normal glucose tolerance during the glucose tolerance, hydrocortisone tolerance and hydrocortisone-glucose tolerance tests. Urinary phosphorus ex-

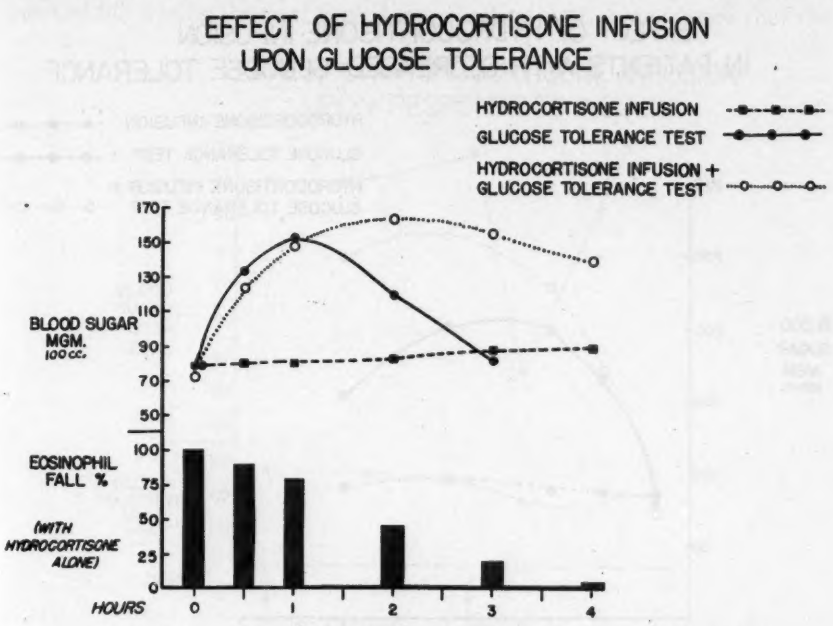

FIGURE 1

cretion was measured in terms of milligrams per hour in 4 patients during the hydrocortisone infusion.

Total blood eosinophil counts were obtained in 6 patients during the four-hour hydrocortisone infusions. The method of Thorn *et al.* was utilized (23). The effect of controlled saline infusion on the blood glucose level and the eosinophil count has previously been shown to be insignificant (6, 7).

Six other patients received similar hydrocortisone infusions as outlined and in addition received 0.1 unit of regular insulin per kilogram of body weight intravenously two hours after the start of the infusion. Serial specimens of blood for sugar determinations were drawn at thirty-minute inter-

vals for the next ninety minutes. Serum phosphorus levels were also determined when possible by the method of Youngberg (24). The procedure was designated as the *hydrocortisone-insulin tolerance test.*

RESULTS

Hydrocortisone tolerance test: Significant variation in blood sugar levels did not occur during the four-hour period of infusion with 100 mg. of hydrocortisone alone in 9 patients with normal glucose tolerance (com-

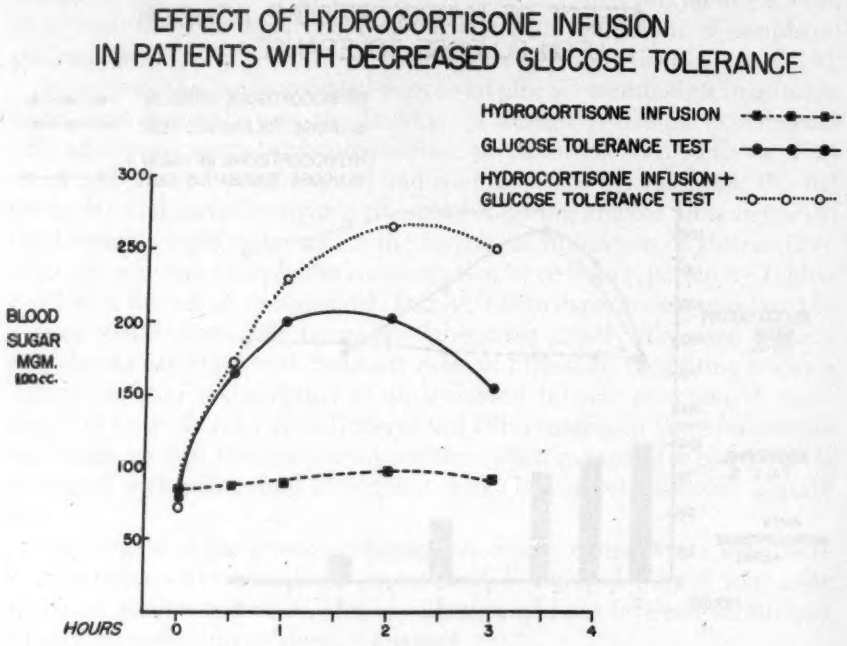

FIGURE 2

posite curve, Fig. 1) and in 3 patients with decreased glucose tolerance (Fig. 2).

Hydrocortisone-glucose tolerance test: Consistent reduction in glucose tolerance was noted in all 8 patients with normal glucose tolerance subjected to this test. This reduction varied and ranged from moderate to marked degree (composite curve, Fig. 1). Comparable results were obtained in patients with decreased glucose tolerance (Fig. 2).

Hydrocortisone-insulin tolerance test: A normal hypoglycemic response to the intravenously administered insulin occurred in the 6 patients, equal to that commonly observed in patients not receiving a prior infusion with hydrocortisone (composite curve, Fig. 3).

Phosphorus studies: The effects of the oral glucose and the hydrocortisone tolerance tests on serum phosphorus levels were comparable. Almost identical moderate falls were achieved by both procedures. However, during the hydrocortisone-glucose tolerance test, profound reductions in the level of serum phosphorus occurred. In 2 patients, the levels fell to 0.6 and 0.7 mg. per 100 cc. respectively at the third hour (composite curve, Fig. 4).

The urinary inorganic phosphorus excretion during the hydrocortisone infusion in 4 patients showed a small rise in the first hour and then a marked fall during the next three hours. In 1 patient, it was noted that the

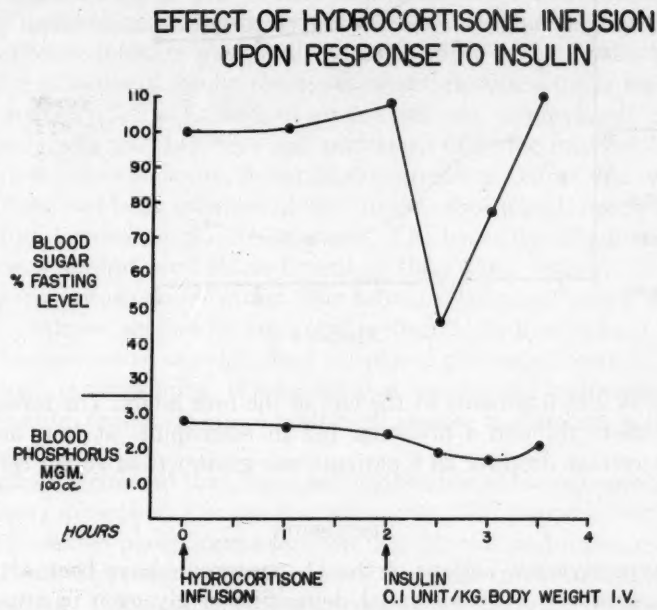

FIGURE 3

urinary phosphorus excretion was 56 mg. per hour for the first hour and fell to 0.7 mg. per hour in the fourth hour. In general, the fall in urinary phosphorus paralleled the changes seen in the levels of serum phosphorus (Fig. 4). This is consistent with the concept of renal clearance mechanisms for phosphorus (25).

The fall in phosphorus levels during the hydrocortisone-insulin tolerance test was comparable to that occurring during the hydrocortisone tolerance test except that following the administration of the insulin, a sharper decline in the level of serum inorganic phosphorus occurred (Fig. 3).

Eosinophils: A significant fall in the blood eosinophil count was noted in the second hour of the hydrocortisone infusion, and the eosinophils dis-

EFFECT OF HYDROCORTISONE INFUSION UPON BLOOD PHOSPHORUS AND URINARY EXCRETION OF PHOSPHORUS

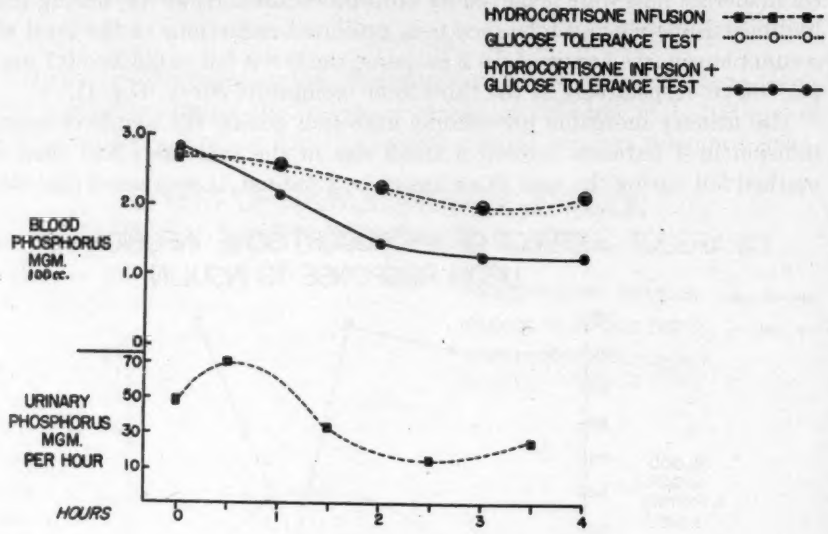

FIGURE 4

appeared in 2 of 6 patients at the end of the four hours. The remaining 4 patients also exhibited a profound fall in eosinophils at four hours, so that the average drop for all 6 patients was greater than 93 per cent (Fig. 1).

DISCUSSION

The hyperglycemic actions of the 11-oxysteroids have been attributed to gluconeogenesis (13), increased deposition of glycogen in muscle and liver (8, 14–16), and decreased peripheral utilization of glucose (2, 3, 17). The failure of the hydrocortisone tolerance test to alter the endogenous blood glucose levels significantly suggests that *at least in the acute experiment*, other mechanisms must be considered. When oral glucose was added to this system, significant decreases in glucose tolerance were consistently achieved.

Falls in the concentration of serum phosphorus during the hydrocortisone tolerance tests were almost identical to those observed during the oral glucose tolerance tests (Fig. 3). Since the fall in serum phosphorus concentration probably indicates peripheral glucose utilization (20), we might assume that glucose is being used peripherally during the hydrocortisone infusion in large enough amounts to maintain the endogenous levels in the

face of possible increased genesis of glucose during the acute experiment. However in the combined procedure, peripheral utilization, though great as measured by the decrease in phosphorus, cannot keep up with the glucose load and glucose tolerance is reduced (Fig. 1). The decrease in glucose tolerance may also be due to inhibition of hexokinase activity, as pointed out by Colowick *et al.* (10).

The marked fall in the blood eosinophils may be taken as evidence that, at least by this physiologic measure, an adequate cortisone response was achieved.

It is of great interest to note that despite the marked effect upon carbohydrate metabolism as shown by the decrease in glucose tolerance, the hydrocortisone infusion was totally without effect in the insulin tolerance test, since evidence of insulin resistance or anti-insulin activity was absent. This is contrary to the findings of other observers, as previously noted (9, 11). The insulin tolerance test was performed after the infusion had been administered for two hours. A significant physiologic effect due to the hydrocortisone had been achieved at this time, as shown by the drop in eosinophils and decrease in glucose tolerance. The hypoglycemic action of the insulin was prompt, and characteristic of the normal subject. The fall in serum phosphorus concentration after administration of insulin is further indirect evidence against an anti-insulin effect of hydrocortisone (Fig. 3). It may be interpreted as evidence of peripheral glucose utilization (20) and, in line with our reasoning, it suggests that insulin and hydrocortisone do not have antagonistic effects on peripheral glucose metabolism in the acute experiment.

It cannot be claimed that the exact significance of the changes in serum and urinary phosphorus is clear at this time. The previous work which showed increased phosphorus excretion (21, 22) was performed with compound E administered by routes other than the intravenous, and over longer periods. The use of intravenous compound F over a four-hour period may explain the difference in our findings. However, the marked fall in the level of serum phosphorus alone may have resulted in a decrease below the renal threshold for phosphorus (25) and hence a failure in continued phosphorus excretion.

The apparent discrepancy between the decreased glucose tolerance and lack of insulin resistance noted during the intravenous infusion of hydrocortisone is suggestive also of other mechanisms. Hydrocortisone may cause the elaboration of an anti-insulin factor, possibly the hyperglycemic-glycogenolytic substance reported to be secreted by the alpha cells of the pancreas (26). This substance may diminish or "neutralize" endogenous insulin activity during a glucose load without appreciably affecting the action of the exogenous insulin used in the insulin tolerance test. Difference

in chemical structure between the exogenous and endogenous insulins may also account for this discrepancy. Hydrocortisone on the other hand, may decrease glucose tolerance by inhibiting the reflex production of increased endogenous insulin elaboration occurring under the influence of induced alimentary hyperglycemia. Hence, although hydrocortisone by itself causes no noticeable change in the concentration of blood glucose, it decreases glucose tolerance under a glucose load.

As with respect to the general endocrine regulation of glucose metabolism (17) or mechanism of action of insulin (27), no one uniform formulation may be offered to account for the changes in glucose metabolism noted under the influence of the 11-oxysteroids.

SUMMARY

The acute effects of intravenous 17-hydroxycorticosterone (hydrocortisone) on glucose tolerance, insulin tolerance, and the level of inorganic serum phosphorus were studied. Infusions of hydrocortisone alone (hydrocortisone tolerance test), hydrocortisone with oral glucose (hydrocortisone-glucose tolerance test), and hydrocortisone with intravenous insulin (hydrocortisone-insulin tolerance test) were administered.

During hydrocortisone infusion alone no hyperglycemic effect was noted, whereas there was a consistent decrease in glucose tolerance during the hydrocortisone-glucose tolerance test. In spite of the decreased glucose tolerance, no anti-insulin effect could be demonstrated.

Profound decreases in the level of serum phosphorus were observed during the hydrocortisone-glucose tolerance tests. Eosinophil falls indicated that active physiologic effects were achieved.

Postulated mechanisms for the changes in glucose metabolism are: 1) increased gluconeogenesis, 2) increased peripheral glucose utilization, 3) secretion of a hyperglycemic-glycogenolytic factor from the alpha cells of the pancreas, and 4) inhibition of reflex secretion of insulin under induced alimentary hyperglycemia.

Acknowledgments

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LACK OF EFFECT OF ASCORBIC ACID ON HYDROCORTISONE METABOLISM IN MAN*

ROBERT C. HAYNES, JR. (CAPTAIN, MC) AND HARVEY L. SHEID (SGT., AMEDS)

U. S. Army Medical Nutrition Laboratory, Fitzsimons Army Hospital, Denver, Colorado

THE work of Bacchus has indicated that in the rat massive doses of ascorbic acid slow the metabolic degradation of administered cortisone, and this inhibition of breakdown prolongs the biologic actions of the cortisone (1–6). Because of the obvious clinical importance, an investigation was undertaken to determine if this effect of ascorbic acid also occurred in man.

EXPERIMENTAL PROCEDURE

Five young men in the age range of 19 to 22 years, who were conscientious objectors to military service, voluntarily acted as experimental subjects. All were in good health at the time of the study.

The metabolism of orally administered hydrocortisone was followed by three groups of determinations: plasma hydrocortisone, by the technique of Sweat *et al.* (7, 8); urinary 17-hydroxycorticoids, by the technique of Reddy *et al.* (9); and urinary 17-ketosteroids, by the techniques of Drekter *et al.* (10) and Vestergaard (11).

The subjects were awakened to void urine at 7:00 A.M. on the morning of the experiment; the sample was discarded. At 8:00 A.M. the subject again voided; this specimen was collected, and blood was drawn into test tubes containing oxalate for determination of the plasma hydrocortisone level. Then the subject was given 50 mg. of hydrocortisone orally. Blood and urine specimens were again collected at 9:00 A.M., noon, 4:00 P.M. and at 8:00 A.M. the following morning. The subjects ate no breakfast on the day of the experiment, but had lunch and dinner. They were kept in bed until 4:00 P.M. and were permitted light activity from then until bedtime.

Five to nine days later the experimental procedure was repeated, but at this time the subjects received ascorbic acid in addition to the hydrocortisone. The ascorbic acid was given on the following schedule: At bedtime the night before the experiment 1 Gm. of ascorbic acid orally; at 8:00 A.M. on the morning of the experiment 2.5 Gm. intravenously and 2.5 Gm.

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intramuscularly; and at 9:00 A.M. 1 Gm. orally. The total ascorbic acid administered was thus 7 Gm. or 84 to 104 mg. per Kg. of body weight. The plasma and urine specimens were frozen until analyses were performed. Analyses of samples for the control day and the day on which ascorbic acid was given were performed simultaneously in order to minimize possible effects of day-to-day variations in analytical techniques.

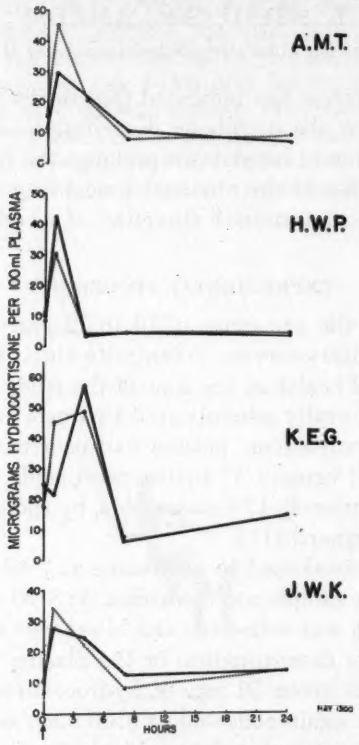

FIG. 1. The level of plasma hydrocortisone expressed in μg. per 100 ml. of plasma. Arrow indicates time hydrocortisone was administered. Values on day hydrocortisone alone was given are represented by a solid line. Values on day ascorbic acid and hydrocortisone were given are represented by a dotted line.

RESULTS

The blood concentration of hydrocortisone rose to a high level within one hour after oral administration of hydrocortisone, and the level returned to normal within eight hours. This is in agreement with the work of Richards and Sweat (12). The changes in plasma hydrocortisone levels on the control day and the day ascorbic acid was given are indistinguish-

able. There was no prolongation of elevated plasma levels, as has been reported in rats, and the height of the curve did not appear to differ significantly from that of the control when ascorbic acid was given. The results of plasma hydrocortisone determinations are given in Figure 1.

The urinary excretion patterns of 17-hydroxycorticoids and 17-ketosteroids did not change significantly when ascorbic acid was given to the subjects. The 17-hydroxycorticoid excretion rate increased shortly after

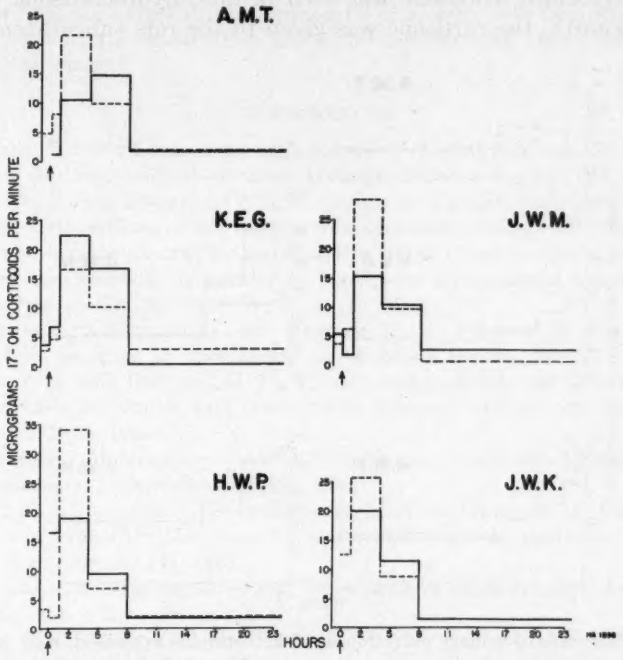

FIG. 2. The rate of urinary excretion of 17-hydroxycorticoids expressed in μg. per minute. Arrow indicates the time hydrocortisone was administered. Values on day hydrocortisone alone was given are represented a by solid line. Values on day ascorbic acid and hydrocortisone were given are represented by a broken line.

administration of hydrocortisone. However, the regular diurnal rhythm of 17-ketosteroid excretion did not seem to be affected by the small dose of hydrocortisone. This is in agreement with the findings of others (13). The excretion of 17-hydroxycorticoids and 17-ketosteroids is shown graphically in Figures 2 and 3.

DISCUSSION

These experiments failed to demonstrate any effect of ascorbic acid on hydrocortisone metabolism in man.

It should be pointed out that there are several differences between the experiments reported here and the experiments of Bacchus, who has reported an effect of ascorbic acid on adrenal steroid hormone metabolism. The first difference concerns species variation; the experiments of Bacchus were performed on rats, and the authors' experiments were performed on men. Second, in the rat studies the ascorbic acid was given intraperitoneally; in these experiments it was given orally, intravenously and intramuscularly. Third, cortisone was used in rats; hydrocortisone was used in man. Fourth, the cortisone was given to the rats subcutaneously; the

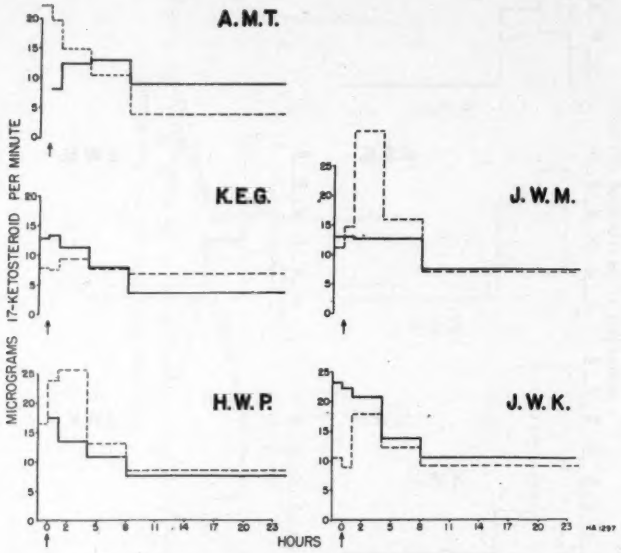

FIG. 3. The rate of urinary excretion of 17-ketosteroids expressed in μg. per minute. Arrow indicates the time hydrocortisone was administered. Values on day hydrocortisone alone was given are represented by a solid line. Values on day ascorbic acid and hydrocortisone were given are represented by a broken line.

hydrocortisone was given orally to the human subjects. Fifth, the dose of steroid was 20 mg. per Kg. of body weight in the rat, but approximately 0.7 mg. per Kg. body weight in man.

Any one of these factors could account for the disparity of the results of the two studies. Which particular variable is the critical one is not known at this time.

SUMMARY AND CONCLUSIONS

Under the conditions of these experiments, massive doses of ascorbic acid given to human subjects failed to affect significantly the metabolism

of a small dose of orally administered hydrocortisone, as measured by changes in plasma hydrocortisone levels and urinary 17-hydroxycorticoid and 17-ketosteroid excretion.

Acknowledgments

We wish to thank the volunteer experimental subjects, The Colorado State Selective Service Organization and Bishop E. M. Yost of the First Mennonite Church of Denver, Colorado, whose cooperation made this study possible.

We also wish to acknowledge generous gifts of ascorbic acid from the William S. Merrell Company, Cincinnati, Ohio, and hydrocortisone from Merck and Company, Rahway, New Jersey.

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EFFECT OF VITAMIN A SUPPLEMENTS ON SERUM PBI LEVEL, AND DISPOSAL OF EXOGENOUS THYROXINE*

T. S. DANOWSKI, M.D., P. WIRTH, M.S., M. H. BLACK, B.S., E. BARTON, B.S. AND R. M. BASTIANI, B.S.

The Department of Research Medicine and the Children's Hospital, University of Pittsburgh, Pittsburgh, Pennsylvania

ADMINISTRATION of vitamin A to animals decreases the size of the thyroid gland (1–3), lowers oxygen consumption (1–4), and partially cancels the hypermetabolic or lethal effects of exogenous thyroxine or desiccated thyroid (1, 5, 6). These findings suggest that in animals excesses of this vitamin may alter thyroid function or interfere with the peripheral effects of thyroxine and related hormones. Several clinical reports of hyperthyroidism responsive to vitamin A or high fat diet are in keeping with both of these possibilities (7–11).

Studies of the possible effects of increased vitamin A intake upon serum protein-bound iodine levels, and upon the disposal of exogenous thyroxine have therefore been undertaken in rats and in man. These indicate that, with sufficient dosage, serum protein-bound iodine concentrations do decrease but that there is no consistent effect upon the disposal of exogenous thyroxine.

MATERIALS AND METHODS

In the first set of studies, levels of serum protein-bound iodine (PBI) were determined in rats and in humans maintained with and without oral supplements of vitamin A. In the animal studies vitamin A acetate[1] was incorporated with ground Purina pellets in amounts sufficient to provide an approximate daily intake of 2,000, 10,000, 20,000 or 30,000 units during periods of eight to twelve weeks. At the end of these intervals blood was obtained by decapitation from these and from control animals and analyzed for serum protein-bound iodine concentrations (12). In the studies with human subjects 4 healthy adults, 2 males and 2 females, received 500,000 units of vitamin A "Anatola"[2] as a daily supplement during a period of

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[1] Vitamin A acetate was kindly supplied by Hoffmann-La Roche under their designation of "dry powder 500–40."

[2] "Anatola"—Parke, Davis & Co. brand of vitamin A derived from fish oil and consisting of esters (approximately 94 per cent) and alcohol (6 per cent).

three weeks. This dosage was selected in view of the demonstration that this amount taken daily can produce anorexia, skin and hair changes, weight loss, headaches, and exophthalmos in human subjects (13–16). Serum was obtained for protein-bound iodine analyses on two successive days of the weeks preceding, concurrent, and following the administration of the vitamin A supplements.

In the second series of studies the disposal of exogenous thyroxine as reflected in the levels of protein-bound iodine was used as an indication of whether or not supplements of vitamin A interfere with the peripheral dis-

TABLE 1. SERUM PROTEIN-BOUND IODINE LEVELS IN FEMALE RATS MAINTAINED WITH AND WITHOUT SUPPLEMENTARY VITAMIN A

Age at start	Duration of study	Approx. vit. A suppl.	Serum PBI (γ/100 cc.)		
	(days)	(units/day)	Mean ± S.D.	Number	"*p*"
(I) Growing (10 wks.)	82–86	2,000	4.2±0.8	(21)	0.68
Growing (10 wks.)	82–86	None	3.8±0.6	(22)	
Grown (18 wks.)	82–86	2,000	4.2±0.8	(23)	0.28
Grown (18 wks.)	82–86	None	3.9±1.2	(20)	
(II) Growing (9 wks.)	84–85	10,000	3.8±0.9*	(26)	0.0045
Growing (9 wks.)	84–85	None	4.5±0.9	(24)	
Grown (14 wks.)	55–57	10,000	3.3±1.1*	(19)	0.018
Grown (14 wks.)	55–57	None	4.1±1.0	(22)	
(III) Grown (15 wks.)	56	10,000	2.9±0.6*	(15)	0.035
Grown (15 wks.)	56	20,000	2.6±0.5*	(20)	0.0003
Grown (15 wks.)	56	30,000	2.3±0.5*	(16)	<0.0001
Grown (15 wks.)	56	None	3.4±0.7	(15)	

* Difference from control mean is statistically significant.

posal of this amino acid. In the animal studies, *l*-thyroxine was incorporated with the ground pellets in amounts sufficient to provide an approximate intake of either 0.2 or 0.1 milligram of thyroxine each day for two to four weeks, with or without supplementary vitamin A acetate, 10,000 units per day. In the studies with healthy young adults a dose of 500,000 units of vitamin A "Anatola" was taken by mouth each day beginning in conjunction with a single dose of oral *l*-thyroxine[3] (0.2 milligram

[3] "Elthrin," the sodium salt of *l*-thyroxine, generously provided by Smith, Kline and French.

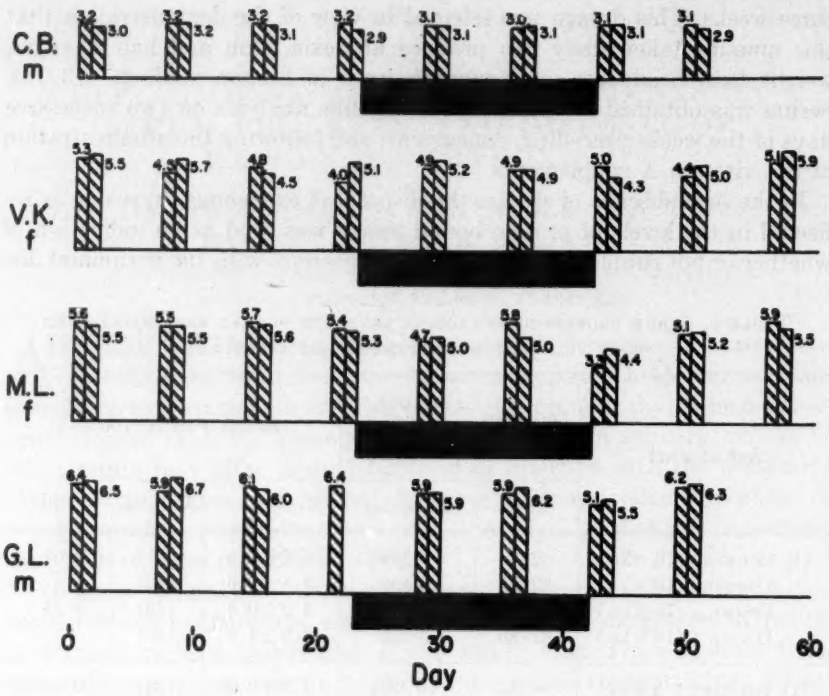

FIG. 1. Effect of vitamin A on protein-bound iodine values (serial determinations in 4 healthy young adults).

The levels (gamma per 100 cc.) during an intake of 500,000 units of Anatola (Parke Davis vitamin A) per day, indicated by the black rectangle, are comparable to those present prior to, and following therapy.

per kilogram of body weight). The rise and the subsequent fall in the level of serum protein-bound iodine induced by the ingestion of *l*-thyroxine during continued vitamin A supplementation were compared with the changes observed with *l*-thyroxine alone.

RESULTS

A. *Effects of vitamin A supplements upon levels of serum protein-bound iodine*

The data in Table 1 indicate that the daily ingestion of 10,000 units or more of vitamin A (approximately 50,000 units per kilogram of body weight) by growing or by grown rats for eight to twelve weeks is associated with a statistically significant decrease in the concentration of serum PBI. This effect was not elicited with a smaller vitamin supplement of approximately 2,000 units per rat, or 10,000 units per kilogram, per day.

The data in Figure 1 indicate that in human subjects also, a comparably low dosage of vitamin A supplement (approximately 10,000 units per kilogram of body weight or 500,000 units per day) given to healthy adults for shorter periods of time did not discernibly modify the level of serum protein-bound iodine. The transient decrease recorded in Subject M.L. coincided with profound and, for this subject, unique dysmenorrhea.

B. *Disposal of exogenous thyroxine with and without vitamin A supplements*

The mean values in Table 2 indicate that in rats a daily supplement of about 10,000 units of vitamin A per day (50,000 units per kilogram of

TABLE 2. SERUM PROTEIN-BOUND IODINE LEVELS IN FEMALE RATS MAINTAINED ON THYROXINE WITH AND WITHOUT SUPPLEMENTARY VITAMIN A

	Age at start (days)	Duration of study	Approx. thyroxine suppl.	Approx. vit. A suppl.	Serum PBI (γ/100 cc.)	
		(days)	(mg./day)	(units/day)	Mean ± S.D.	Number
A.	47	14	0.2	None	17.3 ± 4.4	[23]
	47	14	0.2	10,000	21.4 ± 6.1	[20]
B.	63	28	0.1	None	20.5 ± 2.8	[21]
	63	28	0.1	10,000	17.0 ± 2.4	[18]
C.	56	14	0.2	None	36.9 ± 4.1	[21]
	56	14	0.2	10,000	29.3 ± 4.1	[19]
D.	47	14	0.2	None	25.4 ± 4.9	[20]
	47	14	0.2	10,000	21.8 ± 3.5	[18]

body weight), which was sufficient to lower the serum PBI levels in the first series of experiments, did not uniformly alter the rise in PBI concentrations produced by exogenous thyroxine. In all of the rat experiments the serum PBI values were raised to approximately the same orders of magnitude by the ingestion of thyroxine, irrespective of whether or not supplementary vitamin A was given, and no consistent trend was apparent in the recorded differences.

From Figure 2 it is evident that in humans the effect of oral thyroxine upon the serum PBI level was also not discernibly altered by the daily ingestion of 500,000 units of vitamin A. It should be pointed out that on a body-weight basis, this amount (10,000 units per kilogram) was only one fifth of that employed in the thyroxine studies in rats.

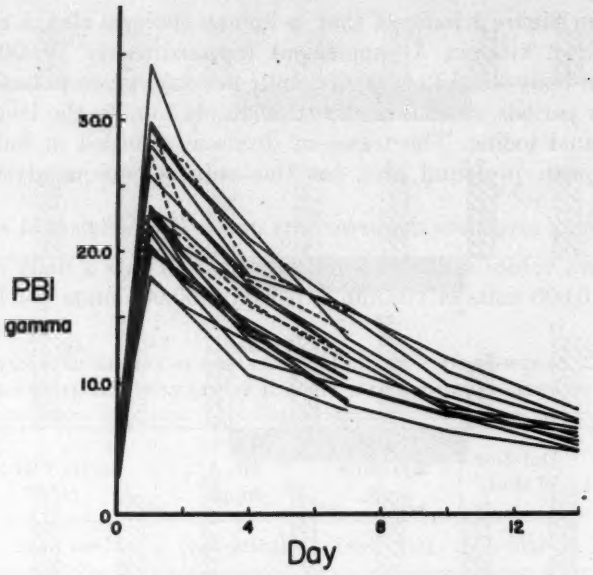

FIG. 2. Disposal of exogenous thyroxine.

Broken lines identify responses of healthy young adults receiving *l*-thyroxine and vitamin A; solid lines represent control observations with *l*-thyroxine only.

The daily ingestion of 500,000 units of vitamin A did not discernibly alter the rate of decrease in the PBI level following a single oral dose of *l*-thyroxine (0.2 mg./Kg. of body weight).

DISCUSSION

The data presented indicate that in rats daily supplements of vitamin A at a level of 10,000 or more units per rat per day induce a slight but definite depression of the serum concentrations of protein-bound iodine. This was not seen with 2,000 units per day. We have no explanation for the discrepancy between our findings and those of Truscott and Sadhu (17), who reported increases in serum PBI values in rats given excesses of vitamin A. It is possible that the vitamin A preparation which they used contained inorganic or organic iodine, which could raise the PBI values (12, 19).

Since the serum PBI level usually reflects the level of thyroid activity (18), our findings point to a decrease in thyroid function under these experimental conditions. This would be in keeping with the depression of oxygen metabolism, the decrease in size of the thyroid, and the lessened thyroidal I^{131} uptake reported by other workers (1–4, 20). Furthermore, these effects of excesses of vitamin A can be taken to be the counterpart of the changes produced by vitamin A deficiency. Under such conditions oxygen consump-

tion has been found to be increased, with concomitant enlargement of the thyroid gland (21–24). The latter change may or may not be influenced by the sex of the animals (21, 23), and has been reported to be associated with decreased formation of thyroxine (23).

It has been suggested that the effect of vitamin A excesses is mediated, in part at least, through suppression of the output or the effects of thyroid-stimulating hormone (2, 3, 25–29), especially since vitamin A has been shown to cancel or minimize thiouracil-induced goiters. Our findings are compatible with, but do not provide the direct support for such an interpretation. Alternative possibilities include decreased binding of thyroxine by serum proteins, or altered peripheral disposal. No data are available regarding the first of these two possibilities. The second is excluded, however, by the failure to demonstrate any consistent differences in the mean levels of serum PBI in rats maintained on supplements of *l*-thyroxine with and without extra vitamin A. Hence the data in the rat studies are compatible with decreased elaboration or secretion of thyroid hormone or with a diminished binding of thyroxine by serum proteins.

The studies in humans indicate that a daily dose of 500,000 units of vitamin A taken for three weeks causes no discernible alteration in the serum PBI level. These findings are in keeping with the results in animal studies, in which comparably low supplements, 2,000 units per day given for longer periods of time, were also without effect. The lack of a vitamin A effect upon thyroxine disposal in the human is also in keeping with the results of studies in animals. It does not, however, represent unequivocal confirmation, since in the human studies only a single dose of thyroxine was used, and on a per-kilogram-of-body-weight basis only one-fifth as much vitamin A was given.

SUMMARY AND CONCLUSION

1. Daily supplements of vitamin A, approximating 10,000 to 30,000 units per rat or 50,000 to 150,000 units per kilogram of body weight, were found to induce a slight but definite decrease in the serum protein-bound iodine level at the end of eight to twelve weeks. This suggests but does not establish a partial suppression of thyroid activity, in keeping with the known decreases in oxygen consumption and gland size induced by excesses of vitamin A and perhaps mediated through a decrease in the output or in the effects of thyroid-stimulating hormone.

2. Smaller amounts of vitamin A (2,000 units per day in rats and comparable amounts in humans) did not alter the serum protein-bound iodine concentration when given for eleven weeks and three weeks, respectively.

3. The effect of exogenous thyroxine upon the serum protein-bound iodine level was not altered by vitamin A supplementation. This suggests

that the depression of serum protein-bound iodine concentration by the vitamin is not attributable to increased peripheral disposal, and that the lower values represent a new steady state as a consequence of alterations in the production, release or transportation of thyroid hormone.

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GROSS AND MICROSCOPIC FINDINGS IN CLINICALLY NORMAL THYROID GLANDS*

JD MORTENSEN, M.D.,† LEWIS B. WOOLNER, M.D. AND WARREN A. BENNETT, M.D.

Section of Surgical Pathology and Section of Pathologic Anatomy, Mayo Clinic and Mayo Foundation, Rochester, Minnesota

BASIC and essential to reliable understanding of the disorders of any bodily organ or system is thorough knowledge of the minute gross and histologic anatomic characteristics of that organ. The pathologic findings observed must then be correlated with clinical observations under various circumstances of health, physiologic alterations and pathologic deviations. In reference to the thyroid gland, such basic pathologic data have been rather slow to accumulate because often the routine necropsy does not include removal of the entire gland. Even when the thyroid is removed, a systematic, minute study of the gland is rarely undertaken as part of the routine examination if the gland appears normal externally or when bisected.

In order, then, to obtain additional basic pathologic data concerning the thyroid gland, the following study was undertaken.

MATERIAL AND METHODS

The thyroid gland was removed en toto from each of 1,000 subjects undergoing routine consecutive postmortem examination in the Section of Pathologic Anatomy of the Mayo Clinic from July 1, 1951 through June 30, 1953. Each gland was weighed to the nearest gram. The intact gland was then palpated and the observations made thereby, with the gland actually in the hands of the examiner, were recorded. The gland was then sectioned by a semiautomatic slicing machine that produced slices 2 mm. in thickness. The completely sectioned gland was then scrutinized for any macroscopic lesion and a sketch was made showing the size, number and

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† Fellow in Surgery, the Mayo Foundation, Rochester, Minnesota. The Foundation is a part of the Graduate School of the University of Minnesota.

distribution of all nodular lesions. Microscopic sections were made of all lesions so discovered and, in addition, a section was taken from a representative portion of thyroid parenchyma. Staining for histologic studies was carried out by the routine hematoxylin-and-eosin technic. The authors each evaluated the histologic sections independently. Findings on the routine complete postmortem examination were reviewed for each case.

Clinical records of each patient were reviewed, particularly in respect to findings in the history, physical examination or laboratory tests which could be related to thyroid disorders. We excluded from this study all patients in whom there was any clinical evidence of abnormality of the thyroid gland (Table 1). Sixty-six of the 1,000 subjects (6.6 per cent) undergoing routine consecutive necropsy were excluded from this study because there was clinical evidence that the thyroid may not have been normal.

TABLE 1. SELECTION OF CASES

	Cases	
Excluded from study		179
Clinical evidence of thyroid disorder	66	
Inadequate clinical evaluation of thyroid	113	
Selected for study (no clinical evidence of thyroid disorder)		821
Total consecutive routine necropsies		1,000

Of the 66 excluded, 44 had clinically palpable nodules in the thyroid gland 6 had clinically diffuse thyroid enlargement, 8 had clinical evidence of hyperthyroidism, 9 were clinically hypothyroid, 4 had taken iodine, 2 had taken thiocyanates, 9 had undergone operations on the thyroid and 7 had taken desiccated thyroid. In addition, 113 subjects were excluded because inadequate clinical examination of the thyroid was recorded. We were left with the thyroid glands from 821 patients who underwent routine consecutive necropsy and who had clinically normal thyroid glands as ascertained by routine general clinical examination. This study deals with the gross and microscopic findings in the thyroid glands from these 821 patients.

FINDINGS

Weight of gland.—The weight of the thyroid gland from these patients with clinically normal glands varied consistently with age of the patients. A scattergram (Fig. 1) shows that although the range of weights in any given age and sex group is rather wide, the great bulk of patients are grouped around an average weight which can probably be considered normal for that age and sex classification. There was no essential difference

in average weights of glands from males compared to those from females (Fig. 2). Similarly, when the patients were classified as to whether their usual places of residence were in a goiter belt or a nongoiter belt, according to the studies of Olesen (1) and of McClendon and Williams (2), the average weight of the thyroid glands was found to vary insignificantly in relation to usual residence of the patient (Fig. 3).

Nodularity.—For the purpose of this study, we considered as a nodule

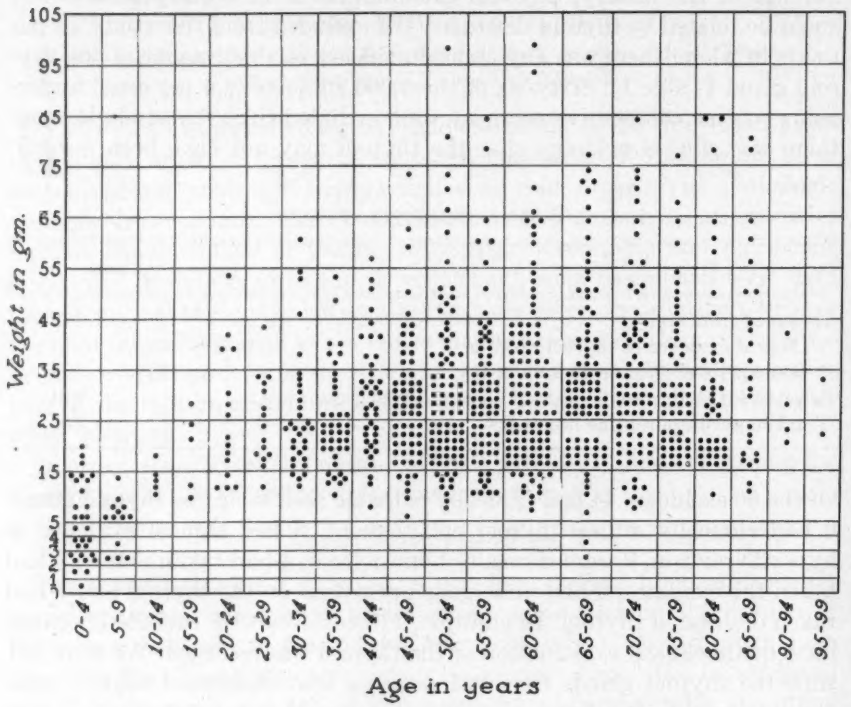
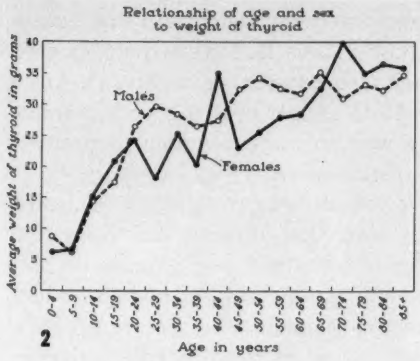
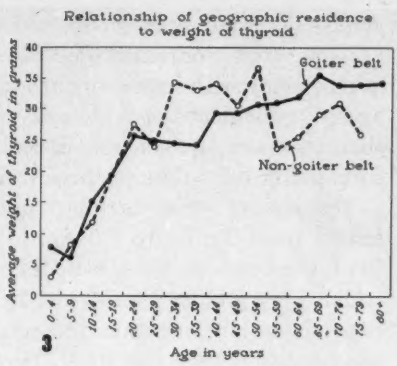
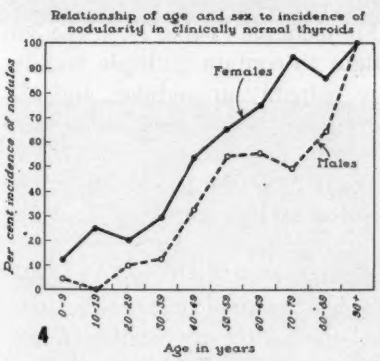


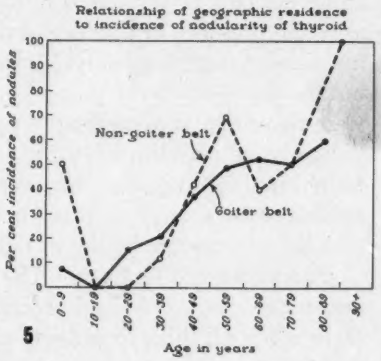
FIG. 1. Scattergram showing relationship of age and sex of patients to weight of thyroid gland.

any discrete macroscopic lesion which could be visually distinguished from the rather homogeneous architecture of normal thyroid parenchyma. Thus neoplasms, involutional or degenerative changes, discrete inflammatory lesions, scars and the like would all be considered nodules so long as they resulted in anatomically discrete, localized lesions that were recognized on macroscopic inspection of the serially sectioned gland. On the basis of these criteria of nodularity, only 415 of these thyroid glands (50.5 per cent) that were clinically normal actually were non-nodular; 100 such









FIGURES 2–5

TABLE 2. INCIDENCE OF NODULARITY IN THYROID GLANDS FROM 821 PATIENTS WITH CLINICALLY NORMAL GLANDS

Thyroid	Nodule detected by				
	Routine clinical palpation (cases)*	Direct palpation of intact thyroid		Macroscopic inspection of serially sectioned glands	
		Cases	Per cent	Cases	Per cent
Non-nodular	821	649	79.0	415	50.5
Uninodular	0	71	8.6	100	12.2
Multinodular	0	101	12.3	306	37.3
Total	821	821		821	

* All patients with clinically palpable nodules were excluded from this study.

glands (12.2 per cent) contained a single nodular lesion and 306 glands (37.3 per cent) contained multiple nodules (Table 2). Nodularity increased in frequency with increasing age in both males and females (Fig. 4). At all ages, the incidence of nodularity was 10 to 20 per cent higher in females than in males. In general, nodularity was no more common in patients from goiter belts than in those from nongoiter belts (Fig. 5).

The size of these nodules, none of which was recognized clinically, varied from 2 mm. to 7.5 cm. in diameter. One hundred and forty-four (35.5 per cent) of the 406 nodular glands contained one or more nodules which were greater than 2 cm. in diameter. Palpability of nodules, which depends on size, location and consistency, seemed to be a more practical consideration than size itself. However, palpation, even with the thyroid gland actually in the hands of the examiner, does not always indicate reliably the presence or absence of nodules. For example (Table 2), of the 306 multinodular glands, only 101 were thought to contain multiple nodules on the basis of direct palpation, 47 were palpably uninodular, and 158 were non-nodular according to palpation. Similarly, of 71 glands that were palpably uninodular, only 24 actually were uninodular, whereas 47 contained multiple nodules. Furthermore, in only 23 of the 100 glands which actually contained a solitary nodule was that nodule correctly identified as a solitary nodule by direct palpation of the gland.

Histopathologic findings.—The pathologic nature of the thyroid nodules encountered was classified according to the histopathologic characteristics. Only when all three examiners agreed that the nodule appeared histologically to represent a true neoplasm was it so classified. Similarly, when all examiners agreed that a nodule appeared histologically to represent inflammatory, involutional, degenerative or physiologic alterations, it was classified as non-neoplastic. Certain nodules could not accurately or unequivocally be placed in one or the other category. In such cases, or when the examiners disagreed as to the histologic diagnosis, the nodules in question were classified as being histologically indeterminate.

Neoplastic and non-neoplastic nodules occurred with about the same general frequency (Table 3). The factors of sex, age, and place of residence of the patient had no demonstrable influence on the incidence of non-neoplastic nodules. On the other hand, interestingly, these factors influenced rather markedly the incidence of neoplastic nodules. Neoplasms occurred considerably more often in females than in males, in patients over rather than under 40 years of age, and in patients living in goiter belts than in those living in nongoiter belts.

The most common nodule observed was the non-neoplastic involutional nodule, variously described with such terms as "adenomatous nodule," "colloid adenoma" or "degenerative-regenerative nodule," which was

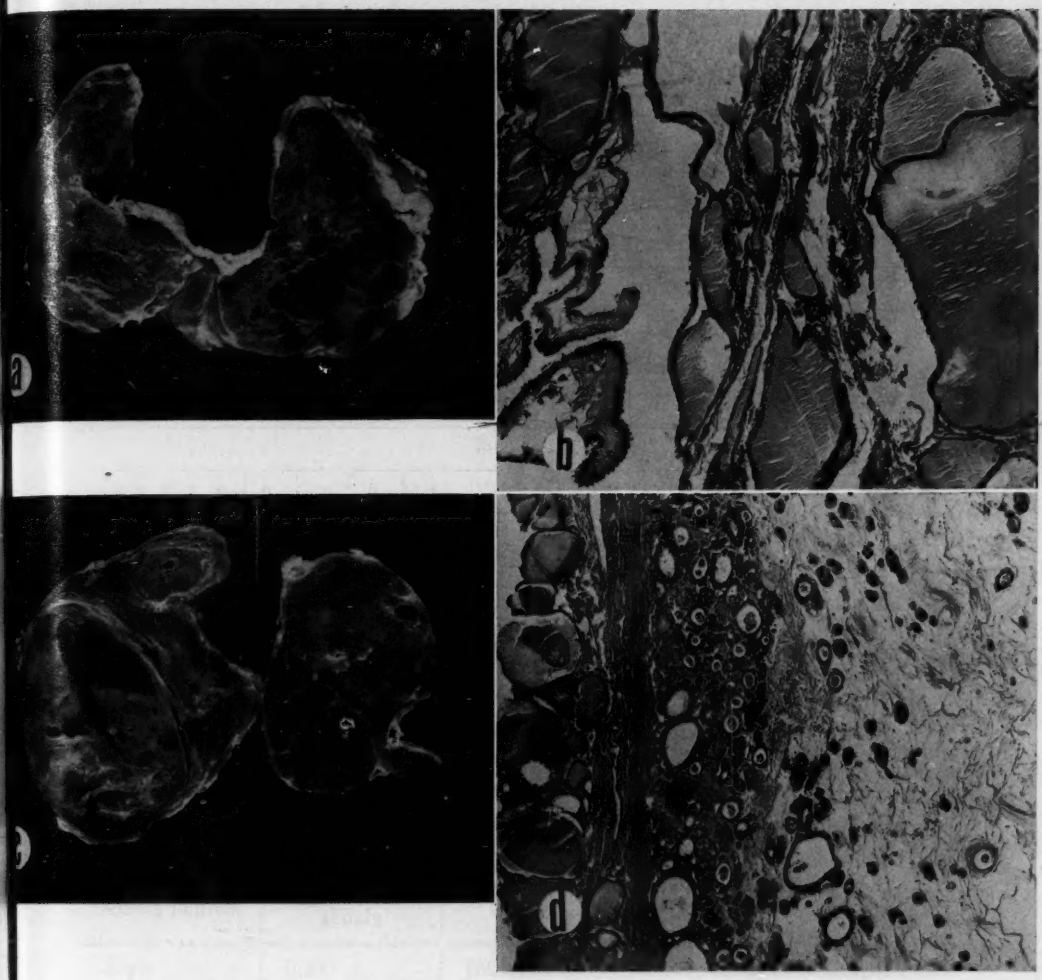


FIG. 6. *Benign thyroid nodules.* **a.** Non-neoplastic macrofollicular colloid nodules. **b.** Histologic appearance of a nodule shown in **a** (hematoxylin and eosin; ×100). **c.** Two benign neoplasms (fetal adenomas). **d.** Histologic appearance of microfollicular fetal adenoma shown in **c** (hematoxylin and eosin; ×100).

present in about a third of all glands studied and in two-thirds of the nodular glands. Other non-neoplastic nodules included focal inflammatory lesions, occurring in about 5 per cent of all glands and in about 10 per cent of the nodular glands studied (Fig. 6 and Table 4).

The second most common nodule was the true adenoma, a benign,

TABLE 3. INCIDENCE AND TYPE OF NODULES FOUND IN THE THYROID GLANDS FROM 821 PATIENTS WITH CLINICALLY NORMAL GLANDS

Age, (yrs.)	Goiter-belt residence							Nongoiter-belt residence						
	Total cases	Nodule						Total cases	Nodule					
		Neoplastic		Non-neoplastic		Indeterminate			Neoplastic		Non-neoplastic		Indeterminate	
		Cases	Per cent	Cases	Per cent	Cases	Per cent		Cases	Per cent	Cases	Per cent	Cases	Per cent
Males														
<20	27	0	0	1	3.7	0	0	2	0	0	1	50.0	0	0
20–49	120	12	10.0	20	16.7	2	1.7	14	2	14.3	5	35.7	0	0
50+	359	168	46.8	158	44.0	23	6.4	36	9	25.0	14	38.9	1	2.8
All ages	506	180	35.1	179	35.4	25	4.9	52	11	21.2	20	38.5	1	1.9
Females														
<20	23	5	21.7	3	13.0	0	0	1	0	0	0	0	0	0
20–49	61	10	16.4	19	31.1	0	0	11	1	9.1	6	54.5	1	9.1
50+	160	110	68.8	104	65.0	14	8.8	7	1	14.3	3	42.9	0	0
All ages	244	125	51.2	126	51.6	14	5.7	19	2	10.5	9	47.4	1	5.3
Total	750	305	40.7	305	40.7	39	5.2	71	13	18.3	29	40.8	2	2.8

usually follicular neoplasm occurring in about a fourth of all glands studied, and in about half of the nodular glands. Malignant neoplasms, including what we have termed "occult carcinoma," were not rare in these

TABLE 4. HISTOLOGIC NATURE OF NON-NEOPLASTIC NODULES FROM PATIENTS WITH CLINICALLY NORMAL THYROID GLANDS

Nodule	No. of glands	Per cent of 821 clinically normal glands	Per cent of 406 nodular glands
Involutional	263	32.0	64.8
Colloid-filled macrofollicle	128	15.6	31.5
Degenerative follicular "adenoma"	135	16.4	33.3
Regenerative nodule	23	2.8	5.7
Inflammatory	42	5.1	10.3
Focal fibrosis (scar)	26	3.2	6.4
Lymphocytic thyroiditis	10	1.2	2.5
Granulomatous thyroiditis	5	0.6	1.2
Fibrous thyroiditis	1	0.1	0.2
Total	305	37.1	75.1

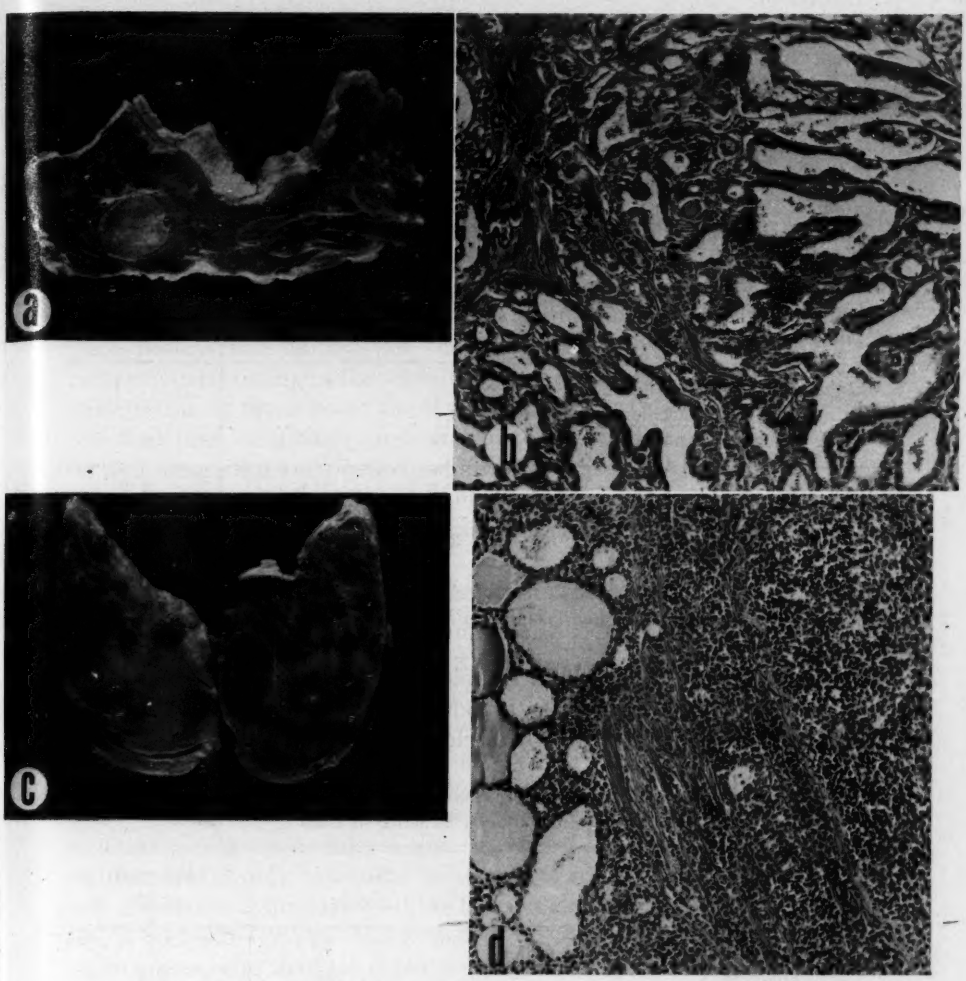


FIG. 7. *Malignant thyroid nodules.* **a.** Solitary discrete nodule, proving to be low-grade, primary, follicular and papillary adenocarcinoma. **b.** Section from nodule shown in **a** (hematoxylin and eosin; ×100). **c.** Lymphosarcoma invading thyroid. **d.** Section from tumor shown in **c** (hematoxylin and eosin; ×100).

patients without clinical evidence of thyroid disorder. Their incidence has been reported in more detail elsewhere (3, 4), but it suffices here to say that, in these clinically normal thyroid glands which contained nodules detected only at routine necropsy, 15 cases of primary "occult carcinoma" of low grade were observed. In addition, there were 2 cases in which a small focus of anaplastic carcinoma was found. The total (17 cases) repre-

TABLE 5. HISTOLOGIC NATURE OF NEOPLASTIC NODULES FROM PATIENTS WITH CLINICALLY NORMAL THYROID GLANDS

Nodule	No. of glands	Per cent of 821 clinically normal glands	Per cent of 406 nodular glands
Benign			
Follicular adenoma	224	27.3	55.2
Papillary adenoma	17	2.1	4.2
Embryonal and solid adenoma	17	2.1	4.2
Hürthle adenoma	14	1.7	3.4
Lipoma	1	0.1	0.2
Malignant			
Primary thyroid carcinoma including "occult carcinoma"*	17	2.1	4.2
Secondary†	15	1.8	3.7
Total	305	37.1	75.1

* Papillary 8, follicular 4, solid 2, Hürthle 1 and anaplastic 2.
† Metastatic carcinoma 10 and lymphoma 5.

sents 4.2 per cent of the nodular thyroid glands (Table 5 and Fig. 7).

Histologic assessment of the internodular parenchyma of these clinically normal thyroid glands (Table 6) disclosed a normal pattern in 83.3 per

TABLE 6. HISTOLOGIC NATURE OF THYROID PARENCHYMA IN PATIENTS WITH CLINICALLY NORMAL THYROID GLANDS

	No of glands		Per cent of 821
Flattened acinar cells and dilated follicles ("hypoactive")		28	3.4
Tall acinar cells		11	1.3
("Focal hyperplasia")	6		
("Diffuse hyperplasia")	5		
"Hürthle changes" in cells		5	0.6
Fibrosis		40	4.9
Focal	36		
Diffuse	4		
Lymphocytic infiltration		53	6.5
Focal	32		
Diffuse (Hashimoto's thyroiditis)	21		
"Normal" thyroid parenchyma		684	83.3

cent of the glands studied. Lymphocytic infiltration and fibrosis each occurred in about 5 per cent of the glands. Histologic findings suggestive of hypofunction of the gland were observed in 3.4 per cent of the cases, and histologic findings suggestive of hyperfunction were noted in 1.4 per cent of cases.

COMMENT

Obviously, a study of this type has definite limitations, which we frankly recognize. Nevertheless, we believe that the objective findings derived from a detailed gross and histologic study of thyroid glands removed at routine necropsy, when correlated with adequate clinical records and complete postmortem findings for the same patient, can and will result in better understanding of the variations of the normal and natural course of various disease processes of the thyroid gland. Furthermore, it would seem essential that such basic gross and histologic pathologic changes should be well recognized and understood if full benefit is to be realized from current improved technics for clinical evaluation, measurement of physiologic variables, and methods of treatment of thyroid disorders.

SUMMARY AND CONCLUSIONS

The results of a detailed gross and microscopic study of thyroid glands removed at routine necropsy from 821 patients with clinically normal thyroids appear to justify the following conclusions:

1. The average weight of the normal thyroid gland depends almost entirely on the age of the patient, and is not consistently affected by the sex of the patient or his usual geographic residence.

2. In about 1 of 8 patients with clinically normal thyroids the gland contains a solitary nodular lesion, in about 3 of 8 it contains multiple nodules, and in only half of the patients is it actually non-nodular.

3. Incidence of nodularity of the thyroid gland increases with advancing age, is higher in females than in males, but is no different for patients living in goiter belts than for patients living in nongoiter belts.

4. Palpation of the thyroid gland often is not reliably indicative of the presence, the absence, or the number of nodules in the thyroid gland.

5. Neoplastic and non-neoplastic nodules occur in the thyroid gland with about the same frequency. Age, sex and usual geographic residence of the patient have little or no relationship to the incidence of non-neoplastic thyroid nodules. On the other hand, neoplastic nodules occur more commonly in females than in males, more commonly in patients over 40 years of age than in those under 40, and more commonly in patients from goiter belts than in those from nongoiter belts.

6. "Occult" primary carcinoma of the thyroid gland occurs about as of-

ten in glands from patients without clinical evidence of thyroid disease but with nodules which are demonstrable pathologically as it does in patients with clinically recognized thyroid nodules, treated surgically—namely 4.2 per cent.

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DEVELOPMENT OF VIRILIZATION DURING PREGNANCY

I. S. FRIEDMAN, M.D., A. MACKLES, M.D. AND I. DAICHMAN, M.D.

The Departments of Medicine, Pathology, and Obstetrics and Gynecology, The Jewish Hospital of Brooklyn, Brooklyn, N. Y.

THE onset of masculinization during pregnancy is extremely rare. Javert and Finn reviewed reports of 122 arrhenoblastomas and found 4 instances in which the onset of symptoms coincided with pregnancy (1). These were cases reported by Felicissimo Paula Xavier and deAbreu Junqueira (2), by Brentnall (3) by Searle, Haines and Baker (4), and later by Falk and Mason (5). The patient in the report by Searle *et al.* had an

TABLE 1. VIRILIZATION DURING PREGNANCY

Author	Onset (month of pregnancy)	17-Ketosteroids (24 hrs.)	Operative findings	Pathologic report
Felicissimo & de-Abreu, 1938	7	—	Single tumor	Arrhenoblastoma
Brentnall, 1945	4	—	Single tumor	Arrhenoblastoma
Baird & Astwood, 1946	4	5th month—8.4 mg.	—	—
Searle, Haines & Baker, 1948	6	Ante partum—158 mg. Post partum—23 mg. Postoperative—13 mg.	Single tumor	Lipid cells and ? arrhenoblastoma
Culiner & Shippel, 1949	8	—	Ovaries enlarged 5x; regressed post partum	—
Falk & Mason, 1951	Antepartum	—	Bilateral tumors	Arrhenoblastomas
Young, 1951	Antepartum	—	Single tumor	Sympathicotropic cells (hilus-cell tumor)
Alexander & Beresford, 1952	4	Post partum—104 mg. Postoperative—3 mg.	Bilateral large tumors	Theca-luteal cells
Friedman, Mackles & Daichman, 1954	5	Ante partum—280 mg. Post partum—4.5 mg. (before operation)	Ovaries enlarged	Bilateral hyperthecosis and rt. dermoid cyst

"adrenal-like" tumor removed, but the other tumors were listed as arrhenoblastomas.

Further instances of the onset of virilization during pregnancy have been published by Baird and Astwood (6), by Culiner and Shippel (7), by Young (8) and by Alexander and Beresford (9) (Table 1). In the first two reports no definitive etiologic diagnoses were possible because oophor-

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ectomy was not performed. In Baird and Astwood's patient, the virilizing symptoms persisted for thirteen years and through three pregnancies in addition to the pregnancy in which virilization was first noted. In Culiner and Shippel's patient the ovaries were noted to be enlarged fivefold at the time of Cesarian section. Eight months post partum the masculinizing symptoms had receded and the ovaries were palpably smaller. Here, too, the ovaries were not removed and therefore the cause of the virilization remains obscure. The patient described by Young had hirsutism for seven years. Her masculinization became very marked as the pregnancy progressed. A sympathicotropic ovarian tumor was removed thirty-nine days prior to the delivery of a normal female child. In Alexander and Beresford's patient, each ovary, five weeks post partum, consisted of multiple cysts the walls of which contained many theca interna cells.

CASE REPORT

E.S., aged 24, first became aware of hirsutism, severe acne, deepening of the voice and enlargement of her nose when she was 22 years of age in the fifth month of her first pregnancy. Her childhood and past history were medically uneventful. Menstruation and libido were also normal. Her father was living and had diabetes mellitus.

The hirsutism, acne and voice changes were progressive but the pregnancy itself remained uncomplicated until the thirty-fifth week of gestation, at which time there was disappearance of the fetal heart sounds. Two days later a stillborn male fetus was delivered spontaneously. The placenta contained multiple areas of infarction. The lungs of the stillborn infant were atelectatic and the alveoli were filled with amniotic fluid. Both testes were undescended but the penis appeared normal.

The results of oral glucose tolerance tests performed several days post partum and again one month after delivery were interpreted as being within normal limits. Several months following delivery the hirsutism disappeared and the voice changes improved, but the skin lesions did not subside for almost a year. Menstruation occurred three months post partum, and remained normal until the next pregnancy.

In April 1953, fifteen months after the first delivery, she became pregnant again without difficulty. On examination during the second month of pregnancy the right ovary was palpable and thought to be about 4 or 5 cm. in diameter; the left ovary was slightly enlarged. During the fifth month of gestation, as in the previous pregnancy, widespread hirsutism developed, associated with severe nodular acne and marked deepening of the voice. During the eighth month of gestation edema of the face, hands, legs and feet was noted. She was now hospitalized for study and induction of labor.

When examined she was found to be a well developed young woman 68 inches tall, weighing 180 pounds. Her skin was oily and her face, chest and back were studded with large nodular and pustular acneiform lesions. Large patches of skin, particularly over the chest, appeared ichthyoid. These roughened areas contained innumerable tiny, white papules which resembled sudamina. There was no abnormal degree of pigmentation. Apart from the pregnancy changes, the breast areolae did not appear unusual. The appearance of her face was startling. It was moon-shaped and covered with nodular pustules. In addition, the face was enveloped by long dark hair suggestive of a beard, mustache and sideburns. The nose was large and edematous. The voice was deeply resonant and low-pitched. No abnormalities of the tongue, eyes, or eyegrounds, thyroid gland,

lymph nodes or heart and lungs were noted. The blood pressure was 130/70. The breasts were of the expected size and were neither tender nor nodular. The uterine fundus was 8 cm. below the xiphoid cartilage. No other abdominal viscera or masses were palpable. The labia minora and majora were thickened and enlarged. The clitoris was 4 cm. long and 1 cm. thick and resembled a small penis. An extensive growth of coarse black hair was also present over the abdomen and lower extremities. There was moderate edema of both legs and feet. She was not obese and there were no abdominal striae.

Roentgen studies showed the heart, lungs, sella turcica and the hands to be normal. An electrocardiogram recorded regular sinus rhythm without any abnormalities. The glucose tolerance test was repeated and the curve was again found to be within normal limits. The level of fasting blood sugar was 80 mg./100 ml., urea nitrogen 12 mg./100 ml., calcium 5.1 mEq./L, phosphorus 2.5 mEq./L, total protein 5.4 Gm. per cent (albumin 3.8 Gm., and globulin 1.6 Gm.) carbon dioxide 19 mM./L, sodium 142 mEq./L, potassium 4.4 mEq./L, chloride 106 mEq./L, and total cholesterol 197 mg./100 ml. (29 per cent free cholesterol). The blood Wassermann and Mazzini reactions were negative. The urine was acid in reaction and had a specific gravity of 1.004; it showed a trace of protein but no sugar; the sediment contained occasional red and white blood cells. The blood hemoglobin value was 12 Gm. per 100 ml., the red blood cell count was 4.2 million per cu. mm., and the white cell count was 7,950 per cu. mm. The blood smear contained 74 per cent polymorphonuclear leukocytes, 20 per cent lymphocytes, 4 per cent monocytes and 2 per cent eosinophils. Four days prior to the induction of labor, the 24-hour urine specimen (1,820 ml.) contained 273 mg. of 17-ketosteroids. This was repeated six days post partum and found to be 280 mg. At this time the pregnanediol content of the 24-hour urine specimen was 85 mg. A month later, five weeks post partum the 17-ketosteroid level in the 24-hour urine specimen had fallen to 13.6 mg. Pregnanediol was absent, as were gonadotropic substances. A trace of estrogen was present.[1]

Labor was induced during the thirty-sixth week. Delivery was uneventful and a female infant weighing 7½ pounds was born. The infant appeared normal, although the labia were slightly enlarged. Within the first three post partum days the patient's edema subsided completely and she lost 25 pounds in weight. No colostrum was present and she did not lactate. Pending uterine involution, further studies and therapy were postponed.

The patient was rehospitalized five weeks post partum (Fig. 1). Edema was absent and the hirsutism had regressed appreciably but the nodulopustular acne remained very marked. Her voice was still deep and the clitoris was three times normal size. As previously, the right ovary was felt to be 4 or 5 cm. in diameter and the left ovary was slightly enlarged. A menstrual period occurred while she was in the hospital. Results of blood chemical studies were similar to those during her previous admission, except that the total plasma protein level had increased to 7.9 Gm. per cent (albumin 5.1 Gm., globulin 2.8 Gm.). The icterus index was 7; cephalin flocculation was normal, and the thymol turbidity value was 2.4 Maclagan units. The alkaline phosphatase level was 2.9 Bodansky units. The serum vitamin A content was 65 μg. per 100 ml. and carotene level, 210 μg. per 100 ml. Results of repeated urine analyses were normal. The blood hemoglobin content remained at 12 Gm. but the red blood cell count had increased to 5.2 million per cu. mm. The white cell count and differential smears were normal. The sedimentation rate was 46 mm. per hour and the hematocrit was 43 per cent. A 24-hour

[1] We are indebted to Dr. A. E. Laufer of The Endocrinology Laboratories of the Jewish Hospital of Brooklyn for these determinations.

urine specimen now contained 13.6 mg. of 17-ketosteroids. Nine days later the level had fallen to 4.5 mg.

When roentgen studies of the abdomen preliminary to intravenous pyelography and retrorectal air insufflation were performed, a right-sided dermoid tumor containing five formed teeth was seen. The pyelograms were interpreted as showing no abnormalities of the kidneys, pelves, ureters and bladder. With retrorectal air studies, normal sized adrenal glands were clearly delineated. Several days later a total right oophorectomy and a subtotal (90 per cent) left oophorectomy were performed. The adrenal glands were palpated and found to be of normal size. The postoperative course was uneventful.

During the following three months the facial hirsutism disappeared. The patient complained that her temporal hair line was receding. The lower half of her body remained hirsute but this, too, had improved. However, a very prominent male escutcheon was still present. Many of the facial nodules had spontaneously drained purulent material and the skin of her back and chest had become soft and smooth in texture, though showing many small residual scars. Her nose had resumed its normal contour and size and her voice had become higher pitched. The clitoris had regressed to normal length. Her infant daughter was alert, well developed, and showed no signs of virilization.

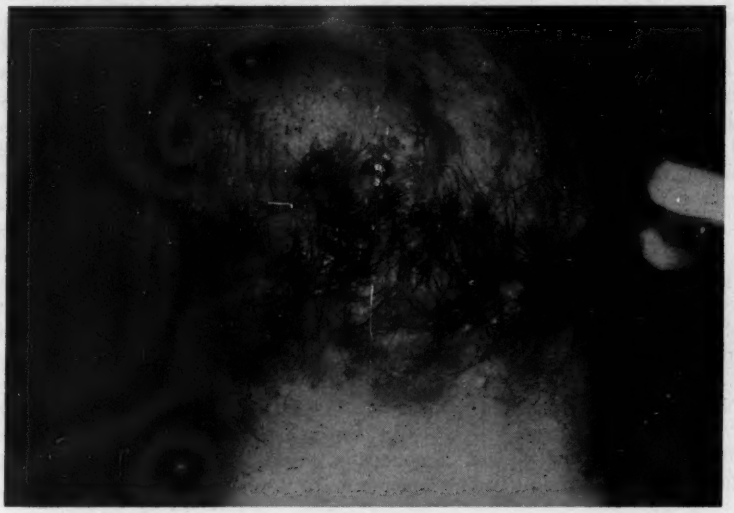

FIG. 1. Patient E.S. Skin of mandible five weeks post partum—hirsutism had appreciably regressed; nodulopustular acne was still marked.

Pathologic Report

Gross description (Fig. 2): The specimen consisted of the right ovary, portions of the left ovary and the appendix. The latter did not appear unusual.

The tissue from the left ovary was composed of several separate portions measuring from 1×1 cm. to 5×1.2 cm. Parts of the surfaces were lined by a smooth and delicate gray capsule. Within the cut surfaces numerous dark brown, well demarcated areas, varying from less than 1 mm. to 4 mm. in diameter were noted. Several contained

pale yellowish gray centers. The greatest concentrations of these areas were beneath the surface capsule and within the cortex of the ovary.

The right ovary measured 9.5×5×5 cm. One portion of this ovary was occupied by a cyst 4.5 cm. in diameter, which contained a cheesy white material and long black hairs. The inner surface of the cyst was smooth and pearly gray and a mound projected into its cavity. Within this mound five tooth-like structures, four of which were close together, could be palpated. A rim of ovarian parenchyma up to 1.5 cm. in width surrounded the cyst on three sides. Within this rim were many small irregular dark

FIG. 2. Gross specimens. Note the many dark lipid nodules in each ovary. The dermoid cyst in the right is clearly seen.

brown spots similar to those seen in the portions of the left ovary. Their size and distribution were also similar.

Microscopic description (Figs. 3–5): Apart from the nodules, sections from the ovaries showed little of note. The components of these dark brown areas were as follows:

1. There were groups and short cords of moderately large cells with delicate, fairly distinct cell borders. These cells contained small, round, deeply staining nuclei with clear cytoplasm containing red-brown granules. The granules were generally fine but in some, groups of cells tended to be large and coarse (Fig. 3). These granules did not stain by the Turnbull Blue method for iron. The cytoplasm of these clear cells contained large amounts of neutral fat as shown by the Scharlach R method of staining (Fig. 4). When

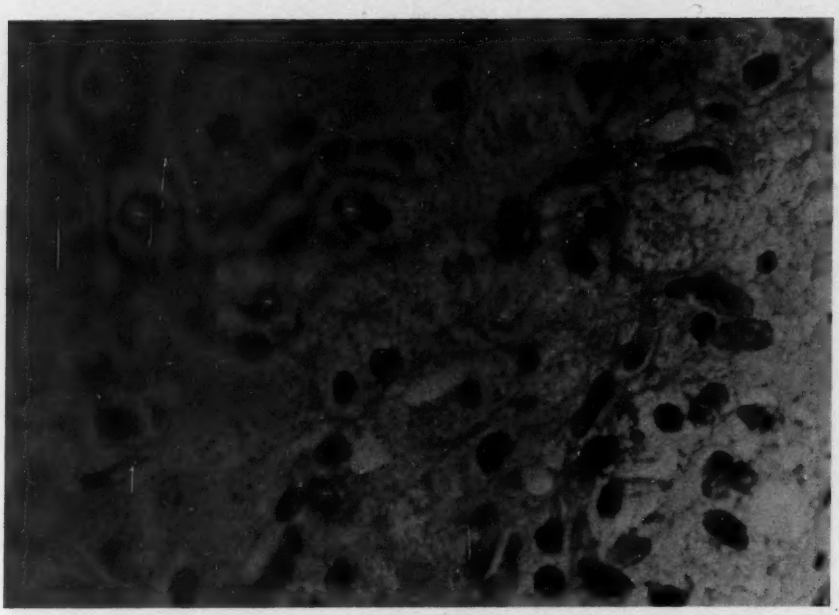

FIG. 3. High-power view of the lipid cells showing cytoplasmic granules. (×460)

stained by the Wilder method, a delicate reticulum was seen surrounding each cell; the cytoplasmic granules did not take the stain.

2. Broad septa composed of hyalinized connective tissue were observed. In most nodules the connective tissue showed no particular distribution with relation to the nodule. However, in several of the smaller nodules the connective tissue was central and

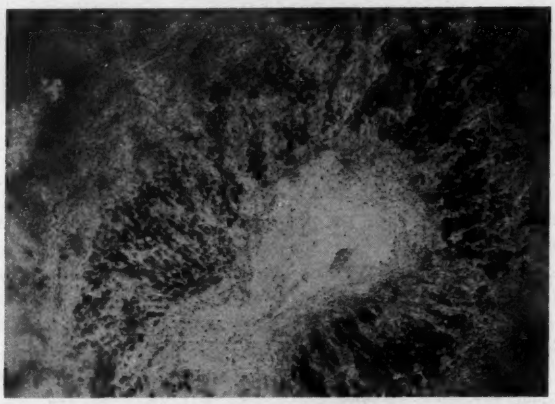

FIG. 4. Scharlach R stain, showing abundance of fat and corpus luteum-like distribution of cells within a nodule. (×75)

surrounded a small irregular cavity containing pink-staining homogeneous material (Fig. 5). There was a marked variation in the proportion of connective tissue and clear cells in the different nodules.

3. Blood vessels the size of small arteries, with thick hyalinized walls, were noted at the periphery of the nodules or just inside the outermost portion. Many of the lumens were wide, whereas others were narrow. In several nodules consisting of relatively few clear cells an abundance of thinner blood vessels was noted.

The largest of these nodules, possibly representing the degenerated corpus luteum of the pregnancy or of the last menstruation, measured 9 mm. in diameter on the slide. Its features were similar to those of the smaller nodules

Sections from the right ovarian cyst showed the mound to be lined by stratified

FIG. 5. A small nodule with a central space, fibrous middle zone, and a periphery of luteal cells. (×75)

squamous epithelium. The core contained fat, sebaceous glands, sweat glands and hair follicles. No adrenal, thyroid, pituitary or gonadal tissue was present.

Pathologic Diagnoses:

1. Cystic teratoma (dermoid cyst) in the right ovary.
2. Right ovary and portions of the left ovary—nodular collections of lipid cells showing evidence of regressive changes.

DISCUSSION

There can be no doubt that the virilization was associated with the ovarian lesions. The normal skull upon x-ray examination, and the subsidence of the symptoms and signs post partum make the presence of a pituitary tumor unlikely. The fact that the adrenal glands were not enlarged to direct palpation at operation and by retrorectal air studies eliminates the possibility of an adrenal neoplasm. The presence of definite lesions in both ovaries, showing varying stages of regression, points to these as the cause of the androgenic activity.

Since the dermoid cyst contained no structures other than teeth, hair, sweat glands and sebaceous glands, it must be regarded as unrelated to the virilization syndrome. In this case, the potentially androgenic cells were endocrinologically active only during pregnancy but nevertheless did not result in early termination of the pregnancy. In other documented cases the functioning of such cells was not related to pregnancy (1). Whether the virilizing activity of these cells was autochthonous or dependent upon pituitary or placental stimulation is unknown.

The excretion of large quantities of 17-ketosteroids in this patient (280 mg. in a 24-hour urine specimen) suggested the presence of an adrenocortical carcinoma (10). These extreme levels, however, helped to rule out considerations of an arrhenoblastoma or adrenocortical hyperplasia since such patients rarely excrete more than 25–40 mg. of 17-ketosteroids per day. Searle, Haines and Baker (4) found that their patient excreted 158 mg. of 17-ketosteroids in a 24-hour urine specimen during the antepartum period. Three weeks post partum the excretion level was 23 mg. and then, after the removal of a cystic ovarian tumor, 13 mg. The cells of this tumor resembled those of the adrenal cortex but could have been of thecal origin. Other observers, however, have interpreted this case as one of arrhenoblastoma with an overgrowth of lipid cells. The patient reported by Alexander and Beresford (9) excreted 104 mg. of 17-ketosteroids on the thirtieth postpartum day. After the removal of bilateral polycystic theca-luteal tumors, normal amounts of 17-ketosteroids were excreted. Our patient, on the other hand, by the fifth postpartum week, prior to operation, excreted normal quantities.

When masculinization results from an ovarian lesion (11–14) the expected findings are either those of a neoplasm [arrhenoblastoma (1); the so-called "adrenal rest tumor" and its many synonyms (15, 16); gynandroblastoma (17–19) or hilus-cell tumor (20, 21)] or hyperplasia of either theca interna cells (7, 22) or hilus cells (20). The fact that the onset of symptoms occurs during pregnancy is of little aid in distinguishing between neoplasia and hyperplasia. Five instances of arrhenoblastoma, "adrenal rest tumor" or sympathicotropic tumor with symptoms during pregnancy have been described, but in none of these cases was there evidence of spontaneous regression of the virilizing features. Of the 3 cases of virilization during pregnancy not demonstrably associated with an ovarian neoplasm, 2 unfortunately did not have histologic studies of the ovaries. In the case reported by Alexander and Beresford (9) the ovaries were removed because of their marked enlargement; both ovaries proved to be polycystic. The lining of the cysts was considered to be luteinized theca interna cells. No mention was made of regression of the virilization during the six weeks between delivery and removal of the enlarged ovaries. How-

ever, the persistent postpartum elevation of the urinary 17-ketosteroids in their patient disappeared after the removal of the theca-luteal neoplasia. In our patient we believe that the diminution of the 17-ketosteroids was due to the regression of the hyperplastic luteinized cells prior to operation.

The closest parallel to the findings in our case is in the case reported by Culiner and Shippel (7). Their patient showed considerable regression of the virilizing symptoms eight months post partum, associated with a significant decrease in the size of the ovaries. One can only speculate that the ovaries of their patient would have shown histologically similar lesions.

In our patient the ovarian lesions are regarded as non-neoplastic. Support for this contention is furnished by the multiplicity of lipid-containing nodules, the bilaterality of the nodules and their small average size, and the regression of the virilization following the termination of the pregnancy. The latter occurred on two occasions. The histologic finding that the cells of the ovarian nodules had clear cytoplasm, roughly central nuclei and fine cytoplasmic granules indicated that they were either of luteal or aberrant adrenocortical origin. In this patient, however, the problem is less difficult when all of the microscopic findings are considered. Here, the largest mass represented the regressed corpus luteum of either the last menstrual period or the pregnancy. Its cells differed in no way from those of the smaller nodules. Some of the smaller nodules showed a central area of fibrosis and a space filled with fluid, whereas the periphery contained cells similar to those of the large corpus luteum. These smaller nodules appeared to represent regressing corpora lutea in which there was a persistence of lipid-filled cells. It would seem then that the clear cells were of follicular or luteal origin, and were not adrenal rests.

Do the lipid-filled cells represent luteinized granulosa cells or luteinized theca interna cells? This question cannot be answered on the basis of histologic findings. There is, however, circumstantial evidence that theca interna cells are capable of elaborating androgenic substances (13); and others have noted the association of hyperthecosis with virilism (22). It would seem that the nodules found in the ovaries of the patient herein reported consisted of luteinized theca interna cells that produced large amounts of androgenic hormone during pregnancy. At the time of removal of the ovaries, these nodules were in a stage of regression featured by extensive inroads of connective tissue into the nodules and the virtual disappearance of some of them.

SUMMARY

A case is reported of a woman in whom virilization occurred during two pregancies and was associated with hyperplasia of ovarian lutein cells.

The virilizing syndrome, including the markedly elevated 17-keto-

steriod excretion, subsided post partum simultaneously with the regression of these hyperplastic cells.

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ABERRANT ADRENAL FUNCTION

JAMES T. BRADBURY, Sc.D. AND WILLIAM G. SLATE, M.B., CH.B.

Department of Obstetrics and Gynecology, State University of Iowa, Iowa City, Iowa

THERE are probably three general types of hormones produced by the adrenal cortex; 1) those controlling electrolyte metabolism (electrocortin or aldosterone), 2) the glucocorticoids (cortisone or hydrocortisone), and 3) the androgenic substances which are excreted as 17-ketosteroids. Normally, ACTH controls the rate of production of the glucocorticoids and the 17-ketosteroids but has little effect on the production of electrocortin. The glucocorticoids are produced quickly and profusely upon administration of ACTH, whereas the 17-ketosteroid response is slower and of smaller magnitude.

Deviations from the normal are usually quantitative in nature. Some of the steroids may be produced in such grossly disproportionate amounts that metabolites, which usually appear only in traces, become the major excretory components. In the adrenogenital syndrome the androgenic substances are predominant, whereas in Cushing's disease the glucocorticoids are in excess.

In the adrenogenital syndrome the defect is primarily in the adrenal, but in Cushing's disease (basophilic adenoma?) the defect is in the pituitary. Normally, the glucocorticoids restrain the pituitary from producing excessive ACTH, but in Cushing's disease the pituitary is not restrained. Physiologically the adrenogenital syndrome and Cushing's disease are separate entities, but in some cases of Cushing's disease the excess androgens (17-ketosteroids) give rise to virilization in addition to the cardinal signs which are due to excessive corticoids.

ADRENOGENITAL SYNDROME AS EVIDENCE OF ADRENAL INSUFFICIENCY

It has been postulated by Jailer (1) that if there is an insufficiency in the production of glucocorticoids, the pituitary increases its output of ACTH until it brings about the production of the daily requirements of glucocorticoids. The increased output of ACTH, necessary to produce normal quantities of corticoids, simultaneously causes an overproduction of 17-ketosteroids or their precursors. This excessive ACTH action on the adrenal thus gives rise to functional or histologic hyperplasia of the cortex. When cortisone is administered to these patients, the substitution therapy eliminates the need for endogenous glucocorticoids; then ACTH produc-

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tion decreases and the output of 17-ketosteroids decreases markedly. Conversely the administration of ACTH causes a disproportionate rise in the output of 17-ketosteroids as compared to that of corticoids. A relative failure in ability to produce glucocorticoids is the basis of postulating that the adrenogenital syndrome is an expression of an adrenal deficiency.

There are instances (especially in infants) of an Addisonian-type deficiency of the adrenal cortex associated with the adrenogenital syndrome. The electrolyte disturbance probably represents a failure of the zona glomerulosa of the cortex, whereas the deficiency in glucocorticoid production is a failure of the zona fasciculata or reticularis. Recent investigations (2) suggest that the growth hormone (STH, somatotropin) may control the glomerulosa, whereas the two inner zones are controlled by ACTH (adrenocorticotropin).

17-KETOSTEROIDS AND ANDROGENS

The 17-ketosteroids in urine are degradation products of several different hormones, some of which are not known. As recovered from the urine, several of the ketosteroids are biologically inert and afford no evidence as to the androgenic potency of their precursors. The routine laboratory methods recover these compounds in a mixture without regard to origin (adrenal, testis or ?ovary) or to androgenic potency. It has been shown that non-androgenic compounds, such as cortisone, may be degraded and excreted to some extent as 17-ketosteroid compounds. Obviously then, the physical evidences of virilization of the patient may afford a much better index of the androgenic activity of the original substances than the laboratory recovery and identification of excreted 17-ketosteroids.

ADRENAL ADENOMA AND/OR CARCINOMA

Adrenal adenomas or carcinomas may produce both corticoid and androgenic compounds and are usually associated with excessive production of 17-ketosteroids. The administration of ACTH does not cause any significant rise in the production of ketosteroids and cortisone therapy does not cause a decrease in output of ketosteroids by these adrenal tumors. These facts indicate that adenomas and carcinomas are autonomous and are not under hormonal control. Therefore, comparison of the urinary output of steroids before and during the administration of cortisone or ACTH makes it possible to differentiate between hyperplastic and neoplastic changes in the adrenal.[1] In this differential study is should be noted that cortisone will

[1] In cases of Cushing's disease (basophilic adenoma?) administration of cortisone will not suppress steroid excretion. A 41-year-old man with the classic manifestations of Cushing's disease was given a trial course of cortisone. His 17-ketosteroid excretion increased from 17 to 27 mg. per day by the fifth day of administration of 200 mg. of cortisone daily intramuscularly. The urinary ketosteroids also increased while he was receiv-

not suppress steroid excretion in Cushing's disease, whereas steroid excretion may increase markedly in response to test doses of ACTH (3).

DEHYDROISOANDROSTERONE AND ADRENAL TUMORS

The earlier studies on steroids in urine from patients with adrenal tumors were summarized in 1945 in a statement that: "the amount of dehyroisoandrosterone excreted is usually greater than the combined amounts of the other ketones. When cortical hyperplasia is present . . . the β-hydroxy-17-ketosteroid fraction (dehydroisoandrosterone and isoandrosterone) is usually, but not invariably, much smaller than when an adrenal tumor is present." (4). These findings have been verified in subsequent reports in which the sulphuric-acid reaction of Dirscherl and Zilliken (5) was modified and used as a simple color reaction for dehydroisoandrosterone (6–8). Compounds of this type give a blue color and are known as blue chromogens. Patterson (6) found blue chromogen in 3 cases of adrenal tumors but failed to find it in 6 cases of prepubertal virilism with elevated 17-ketosteroids. Allen *et al.* (7) found blue chromogen in 5 cases with adrenal neoplasms but not in 6 cases of female pseudohermaphrodism.

Wolfson (9) has pointed out a fallacy in the assumption that blue chromogen determinations are an accurate measure of dehydroisoandrosterone. Earlier studies of Dobriner and Lieberman revealed that the usual methods of hydrolysis with hydrochloric acid produce artifact substances which still react like dehydroisoandrosterone. Bitman and Cohen (10) designed a method of hydrolysis which recovered dehydroisoandrosterone more quantitatively. Blue chromogen has been found in the urine of normal adults (10) and premature infants (11), so its presence is not specific for adrenal tumor. Even though dehydroisoandrosterone may be found consistently in cases of adrenal tumor it is also present in occasional cases of adrenal hyperplasia. When present in hyperplasia, the dehydroisoandrosterone, along with the other ketosteroids, decreases or disappears when cortisone is administered.

CASE REPORT

The foregoing comments are pertinent to a case study.

The patient was a single 22-year-old female, who complained of amenorrhea when first seen in October 1951. The patient began to menstruate at the age of 12 years. For about a year she had regular 4-day periods at intervals of twenty-eight to thirty days; then they became irregular and ceased at the age of 14. She consulted a physician, who

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ing test doses of 50 mg. of cortisone per day. Subsequently he was adrenalectomized and the histologic diagnosis was bilateral adrenal hyperplasia. In this case the hyperplasia was secondary to a refractory pituitary from which the excess ACTH was causing the adrenals to produce excessive corticosteroids as well as ketosteroid precursors. The exogenous cortisone was metabolized and excreted in part as 17-ketosteroids, thus adding to his daily excretion.

gave her thyroid and estrogen injections, about one a week, for three weeks. He repeated this treatment for several months. These provoked occasional episodes of vaginal bleeding, but no return to spontaneous menstrual periods. She had never had dysmenorrhea. At the age of 13 to 14 years she noted excessive hair growth on the chin and upper lip, chest, lower abdomen, lower back, and the extremities. Epilation was performed on the face during her seventeenth and eighteenth years. She had had occasional treatments since then. Her weight had ranged from 135 to 138 pounds for seven years and she had always been in good health. Her one sister, who is alive and well, had no evidence of hirsutism.

On physical examination, the patient was a muscular brunette female with some light scarring on the face caused by the previous epilation. Some coarse hair was still present on the chin, upper lip, neck, and between the breasts. Axillary hair was heavy. There was accentuation of the hair growth on the abdomen, extending above the umbilicus. Pubic and perineal hair was heavy. Coarse hair was present on the arms and legs. The thyroid was not enlarged. Her blood pressure was 120/75. She had a normal feminine voice. The breasts were fairly well developed. Pelvic examination revealed no enlargement of the clitoris and the genitalia were normal except for a small uterus. The ovaries were not enlarged. No other physical abnormality was detected.

Cyclic estrogen therapy was tried for eight months. The dosage was increased each month until 50 mg. of stilbestrol per day was employed in the eighth trial. There was some progressive growth and tenderness of the breasts during these courses of estrogen therapy but no episode of uterine bleeding was induced. Desiccated thyroid was given in a dose of 1 grain daily for the first month and 2 grains daily in the subsequent months. The pelvic findings remained unchanged. A sound could be passed up the uterus to a depth of 6 cm. An endometrial biopsy (June 1952) made thirteen days after completion of stilbestrol therapy revealed the presence of an inactive endometrium.

A trial of natural estrogen was decided upon, and Premarin (1.25 mg. three times daily) was given for twenty-one days, followed by a rest period of seven days. This was repeated once, but neither course induced bleeding. Five weeks following the cessation of the second course of Premarin, the patient experienced a spontaneous menstrual flow which lasted four days. Twenty-eight days later the patient experienced another spontaneous menstrual period, which again lasted for four days. She noted no breast changes. Cramps did not occur with the menstrual flow. A third spontaneous menstrual flow occurred after twenty-eight days. The only medication taken at the time these menstrual periods occurred was thyroid, 2 grains daily. Thyroid medication was discontinued in November 1952, twenty-six days after the third menstrual period. At this time urinary 17-ketosteroids were 190 mg. per twenty-four hours. A second specimen obtained on December 2 was found to contain 139 mg. per twenty-four hours. The Allen (7) test for blue chromogens on these specimens showed that 75 to 80 per cent of the ketosteroids reacted like dehydroisoandrosterone. These laboratory findings suggested the possibility of an adrenal tumor, so additional clinical studies were made.

Intravenous pyelograms showed prompt bilateral normal renal function. There was no displacement of the kidneys. Perirenal air studies failed to reveal any radiographic evidence of adrenal tumor. X-ray examination of the skull showed the sella turcica to be normal. In a glucose tolerance test the fasting blood sugar level was 106 mg. per 100 cc. and the response to 50 grams of glucose orally was normal. Plasma protein-bound iodine concentration was 7.0 micrograms per 100 cc. (normal value 3.5–8.0 micrograms per 100 cc.).

The finding of 17-ketosteroid 24-hour excretion levels of 190 mg. and 139 mg., to-

gether with a positive blue chromogen (Allen) reaction, led to a tentative diagnosis of adrenal tumor. Even though perirenal air studies failed to provide any radiographic evidence of adrenal enlargement, the patient was scheduled for an exploratory operation. However, she was given 100-mg. doses of cortisone intramuscularly for five days in order to determine the effect on 17-ketosteroid excretion. The prompt fall in ketosteroid values was typical of that reported for cases of adrenal hyperplasia. It was then decided to postpone any exploratory operation until further studies and observations had been made. The patient's weight during the period of observation remained between 135 and

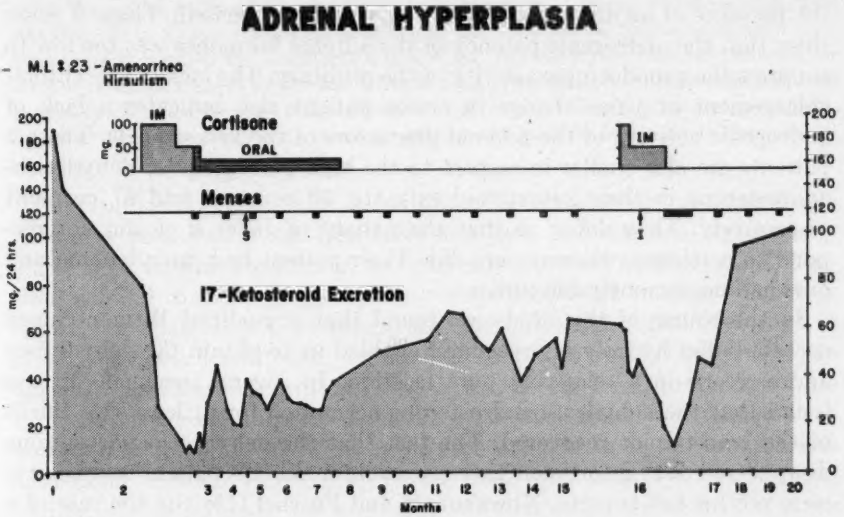

FIG. 1. Graphic representation of 17-ketosteroid excretion values, menstrual rhythm and cortisone therapy during a 19-month observation interval. The time scale is varied to permit recording more frequent ketosteroid values during cortisone tests in the second and sixteenth months. The arrows indicate endometrial biopsies—S for secretory endometrium and I for inactive.

140 pounds. No significant change was noted in hair growth. The dosage of cortisone and the ketosteroid findings are summarized in Figure 1.

February 1953: Endometrial biopsy—an early secretory type of endometrium.

May 1953: Perirenal air studies showed that both adrenals had a normal outline.

February 1954: Endometrial biopsy—inactive endometrium.

There was still no clinical evidence of adrenal tumor.

DISCUSSION

In the management of adrenal hyperplasia it has been suggested (Wilkins *et al.* (12)) that the dose of cortisone be determined as the amount necessary to maintain 17-ketosteroid excretion at 8 mg. or less per day (in adults), and to allow for regression of hirsutism and other signs of

virilism. On this basis, probably the maintenance dose of cortisone would have been 50 to 75 mg. per day for our patient. This was not attempted and the hirsutism did not change visibly. It is of interest, however, that for a year after cessation of cortisone therapy she continued to have a a normal menstrual rhythm even though the 17-ketosteroid excretion values ranged from 50 to 100 mg. daily. Jailer *et al.* (13) noted a normal menstrual rhythm in a patient with 17-ketosteroid values of 50 to 68 mg. per day. In that instance, hirsutism was the only clinical manifestation of the presence of an adrenal adenoma, subsequently excised. These 2 cases show that the androgenic potency of the adrenal hormones was too low to suppress the gonadotropic activity of the pituitary. The lack of any clitoral enlargement or voice change in either patient also indicates a lack of androgenic activity of the adrenal precursors of the ketosteroids. These 2 patients are also similar in respect to the high percentage of dehydroisoandrosterone in their ketosteroid extracts, 50 per cent and 67 per cent respectively. They differ, in that the patient of Jailer *et al.* did not respond to cortisone, whereas ours did. Their patient had an adenoma and ours had no demonstrable tumor.

In the course of this study we found that a modified Bitman-Cohen acetate-buffer hydrolysis procedure[2] enabled us to obtain the dehydroisoandrosterone in a relatively pure fraction. In several specimens it was found that the dehydroisoandrosterone accounted for at least two thirds of the ketosteroids recovered. The fact that the dehydroisoandrosterone disappeared during cortisone therapy would make this patient unique, if it were not for two reports. Nowakowski and Puschel (15) cite the case of a 25-year-old man with adrenal hyperplasia in whom dehydroisoandrosterone was reduced to normal (1 to 2 per cent of the total ketosteroids). Patterson and Swale (16) report that cortisone caused a greater decrease in blue chromogens than in the total ketosteroids in adrenal virilism.

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[2] Jensen and Tötterman (14) found that the initial extraction with butanol as carried out by Bitman and Cohen was not necessary. The urine specimens were adjusted to pH 4.7 with acetic acid and then heated in a water bath at 100° C. for three to four hours. After this mild hydrolysis, extraction with toluene recovered the dehydroisoandrosterone in relatively pure form. This mild hydrolysis is rather specific for steroid sulphates. The urine was then acidified by adding 10 volumes per cent of concentrated hydrochloric acid and boiled for fifteen minutes. The second toluene extraction recovered the remaining ketosteroids which had probably been excreted as glucuronides. In this patient the total yield of ketosteroids was about 25 per cent higher after fractional hydrolysis than after the usual hydrochloric-acid hydrolysis. This is in agreement with the findings of Jensen and Tötterman. In other patients with a low proportion of pH 4.7 hydrolyzable steroids we have not found any appreciable increase in yield by the use of the two-stage hydrolysis.

The experience of Willard Allen and others justifies attempts to demonstrate an adrenal tumor whenever abnormal amounts of dehydroisoandrosterone are found. However, if the administration of cortisone reduces the dehydroisoandrosterone to normal along with the other ketosteroids, the evidence favors the diagnosis of adrenal hyperplasia.

This response to cortisone seems valid evidence of hyperplasia, even when there is radiographic evidence of adrenal enlargement.

Mrs. M. T., aged 30 years, was admitted for evaluation of hypertension, present since the age of 14. She had generalized body hirsutism. Urinary 17-ketosteroids were elevated (15 to 20 mg. per twenty-four hours) but there was no blue chromogen. Cortisone therapy reduced the ketosteroids to 4–6 mg. per twenty-four hours. Perirenal air studies revealed a mass in the left adrenal area. The enlarged adrenal was removed surgically and the pathologic diagnosis was adrenal hyperplasia. On postoperative follow-up after cessation of cortisone therapy, urinary ketosteroids had risen to the original elevated levels.

The prevailing tendency to consider 17-ketosteroids as androgenic hormones is unwarranted. The foregoing case, and the one reported by Jailer *et al.* in which hirustism was the only physical evidence of virilization, showed that the ketosteroids or their precursors were so effectively eliminated that they were practically inert. Dehydroisoandrosterone, a biologically active androgen, was identified in both cases. The fact that in our case it was excreted in an easily hydrolyzable form, probably as the sulphate, suggests that the dehydroisoandrosterone was excreted so quickly that blood levels were never high enough for it to exert marked androgenic effects. Hertz (17) found that intravenously administered sodium estrone sulphate was so quickly and completely eliminated from the blood that it had little systemic effect. Furthermore, sodium estrone sulphate has little estrogenic effect when given orally (18). If future studies show that some of these patients have the ability to conjugate and excrete ketosteroids as well as estrogens as sulphates, such results would offer an explanation as to how steroid hormones may be rendered biologically ineffective.

SUMMARY

In the course of study and treatment of a patient with adrenocortical hyperplasia several unusual features were observed:

1. Dehydroisoandrosterone was identified and found to constitute 67 per cent of the total ketosteroids recovered.

2. The dehydroisoandrosterone was apparently excreted as the easily hydrolyzable sulphate.

3. The diagnosis of adrenal hyperplasia was based on the fact that ketosteroid excretion was reduced from 140–190 mg. per day to the normal level by administration of cortisone.

4. Menstrual rhythm was maintained during a year of observation, even though total ketosteroid excretion was 50 to 100 mg. per day.

5. A possible mechanism for the rapid elimination or "inactivation" of the ketosteroids is discussed.

Acknowledgments

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Teaching Clinic

ADDISON'S DISEASE AND PEPTIC ULCER

RECENT reports of the occurrence of peptic ulcer and its complications in patients under treatment with ACTH or cortisone have led to the concept that the adrenocortical steroids play a fundamental etiologic role in this disease. Gray and co-workers have found that the administration of ACTH or cortisone for from seven to fourteen days resulted in a marked increase in basal and nocturnal gastric secretion of hydrochloric acid and pepsin and in the urinary excretion of uropepsin (1–4). Patients with Cushing's syndrome likewise exhibited an increase in gastric secretion and uropepsin excretion, whereas those with Addison's disease and hypopituitarism had low gastric activity. Furthermore, Gray and associates also noted that both acute physical and chronic emotional stress induced similar changes and that, in general, these changes were not dependent on intact vagus nerves. From such studies they concluded "that emotional stress and anxiety may also *induce* gastrointestinal ulceration by a hormonal mechanism through the hypothalamic-pituitary adrenal-gastric axis" (1). Such a concept implies a direct role of adrenal steroids in the causation of peptic ulcer, as indicated by Gray's statement "these studies indicate that these adrenal steroids stimulate the gastric glands to secrete a significant increase in acid and pepsin and may induce severe epigastric pain, cause reactivation, perforation or hemorrhage of pre-existing peptic ulcer or new formation of ulcer" (3).

This point of view is in disagreement with the concept of the adrenal-stress relationship proposed by Ingle in which the adrenal steroids are considered to play a permissive role, being necessary for, but not directly the cause of certain features of the stress response (5, 6). This concept has been extended by us to include the possibility that excess adrenal hormone sensitizes the organism to respond more readily and/or excessively to certain stressful stimuli (7, 8). A similar interpretation recently has been proposed by Selye in his concept of stressful stimuli conditioning the organism to respond to adrenal hormone (9).

In light of these differing points of view, the observation of a patient with Addison's disease in whom a gastric ulcer developed during maintenance therapy with 25 mg. of cortisone acetate daily is of considerable interest for the interpretation of the relationship between the adrenal gland and peptic ulcer.

CASE REPORT

I.M. (Duke No. D-37259). The patient was a 49-year-old Negro housewife who was admitted to the hospital on April 14, 1952 complaining of vomiting and epigastric soreness for five and a half months. Prior to the presenting illness, she had enjoyed good health. In November 1951, coincident with an upper respiratory infection, she first began to experience morning nausea and vomiting and in time these gradually increased so that she vomited after almost every meal. There was marked anorexia, weakness and a 40-pound weight loss. Coincident with these changes the patient and her family became aware of gradually increasing pigmentation of her face, hands, elbows, knees, and various pressure points. Four months prior to admission she complained of arthralgia without visible swelling or redness of the joints. Three weeks before, she had been admitted to another hospital where she received intravenous infusions which had no significant effect. There she experienced presyncopal sensations on getting out of bed. For several years, she has noted a decrease in the rate of growth of her axillary hair; it had not required shaving for at least two years. Her blood pressure has always been described as low. Past and family histories were not contributory.

Physical examination revealed a middle-aged woman who was weak, appeared chronically ill, and had obviously lost considerable weight.

Her temperature was 37° C., pulse 80, respirations 16, blood pressure 100/76 mm. Hg and weight 134 pounds. The skin of the face and neck exhibited a dirty irregular type of pigmentation which stopped at the hair line. There was similar pigmentation over the elbows, knees, hands, nipples, shoulders and lateral thighs. The lids were deeply pigmented. Buccal pigmentation typical of her race was present. Axillary and pubic hair were sparse. The heart and lungs were clear. The liver was palpable one fingerbreadth below the costal margin. There was generalized muscle weakness, but the neurologic examination otherwise showed nothing abnormal.

On the morning after admission the patient's blood pressure fell to 80/60 mm. Hg. An ACTH eosinophil test showed an initial count of 155 cells per cu. mm.; half an hour and four hours after 20 units of ACTH intramuscularly the counts were 144 and 188 cells per cu. mm., respectively. The clinical impression was Addison's disease and hence therapy was initiated with intravenous dextrose and saline, 25 mg. of cortisone acetate every six hours by mouth, and 10 mg. of desoxycorticosterone acetate (DCA) intramuscularly. By the sixth day, hormone dosage had been reduced stepwise to 12.5 mg. of cortisone twice a day and 2.5 mg. of DCA daily. The patient responded dramatically to this treatment with an increase in strength, a rise in blood pressure, disappearance of nausea, vomiting and anorexia and a general change in her mental attitude. Most diagnostic procedures were deferred until cortisone therapy was discontinued after two weeks in the hospital.

Laboratory and special studies revealed the following: Hgb. 12.8 Gm., RBC 4,100,000 per cu. mm., hematocrit 37 vol. per cent, WBC 5,400 per cu. mm., with 58 per cent polymorphonuclear leukocytes, 32 per cent small lymphocytes, 6 per cent large lymphocytes, and 4 per cent monocytes. Ten days after initiating treatment the hemoglobin was 10.5 Gm., and the hematocrit 29 vol. per cent. Results of urine and stool examination were negative. The tuberculin (1:10,000) reaction was positive. The value for fasting blood sugar was 80 mg. per 100 ml., nonprotein nitrogen 52 mg. per 100 ml., CO_2 combining power 30 mEq. per liter, sodium 143.3 mEq., chloride 94.3 mEq., and potassium 4.3 mEq. per liter. Gastric analysis showed no free acid before or after histamine. Results of an insulin-glucose tolerance test on April 22, 1952 (while the patient was receiving 25 mg. of cortisone) were: blood sugar level 79 mg. per 100 ml. fasting; 42 mg.

thirty minutes after insulin; 46 mg. thirty minutes after glucose; 57 mg. one hour after insulin; 91 mg. two hours after; and 88 mg. per 100 ml. three hours after insulin. On April 28; twenty-four hours after discontinuing cortisone, results of an insulin-glucose tolerance test were: blood sugar level 69 mg. per 100 ml. fasting; 29 mg. half an hour after insulin; and 39 mg., 53 mg., 57 mg. and 75 mg. per 100 ml. at half, one, two and three hours after glucose. This curve is typical of Addison's disease. On x-ray examination, the chest, abdomen, esophagus, stomach, duodenum and small bowel were normal (Fig. 1.). There was no adrenal calcification visible in the abdominal x-ray film.

A 125-mg. pellet of desoxycorticosterone acetate was implanted and the patient was discharged taking 12.5 mg. of cortisone acetate twice daily. One month after discharge her weight was 151.5 pounds without edema, B.P. 120/80, and hematocrit 42.5 vol. per cent. She felt very well and returned to work. One year later, she had reached a peak weight of 190 pounds and her hematocrit was 48 vol. per cent. A marked decrease in pigmentation was noted. In July 1953, she began to complain of a diffuse burning sensation in the abdomen, associated with nausea but relieved by eating. A gastrointestinal x-ray examination on August 4, 1953 showed a small hiatus hernia but no other abnormality. In January 1955, she again returned with the complaint of a persistent burning epigastric pain of two weeks' duration, occurring during fasting and at night and relieved by Alka Seltzer or eating. An x-ray film revealed a small prepyloric ulcer (Fig. 1). A gastric analysis showed no free acid and 29 units of free acid after histamine. She was admitted to the hospital for study of her adrenal function and the nature of the peptic ulcer. Cortisone therapy was discontinued and in its place a dose of 2 mg. of DCA was administered intramuscularly daily during the eight days in the hospital. The pertinent findings with respect to adrenal and gastric function were as follows: ACTH-eosinophil test (25 units i.v. over eight hours)—77 cells per cu. mm. before, and 40 per cu. mm. after ACTH. Results of a Kepler-Power water test were positive. Urinary 17-ketosteroids were 4.0 mg. per twenty-four hours. Urinary 17-hydroxycorticosteroids (Reddy) were 2.9 mg. per 24 hours. After an 8-hour intravenous infusion of 25 units of ACTH, 17-hydroxycorticosteroid excretion was 2.3 mg. per twenty-four hours.[1] Gastric analysis showed no free acid fasting and 30 units at forty minutes after histamine. Serial x-ray films of the stomach showed progressive healing, with a normal stomach being reported on February 15, 1955, six weeks after discovery of the ulcer and initiation of therapy with diet, antacids and Banthine (Fig. 1). The patient has been asymptomatic since this time and again is receiving 12.5 mg. of cortisone twice daily.

DISCUSSION

The sequence of events in this patient presents a convincing argument for a permissive role of the adrenal cortex in the etiology of peptic ulcer. The patient had prominent gastrointestinal symptoms early in the course of her Addison's disease, as do many patients with this malady, but gastrointestinal x-ray examination revealed no abnormality. Gastric analysis showed no free acid during fasting, and no response to histamine. The patient exhibited an excellent therapeutic response to 12.5 mg. of cortisone acetate twice a day, becoming completely rehabilitated. However, three years after initiating therapy gastrointestinal symptoms developed and a

[1] This test was performed in April 1955, while the patient was receiving 1 mg. of fluorohydrocortisone daily beginning forty-eight hours before the test.

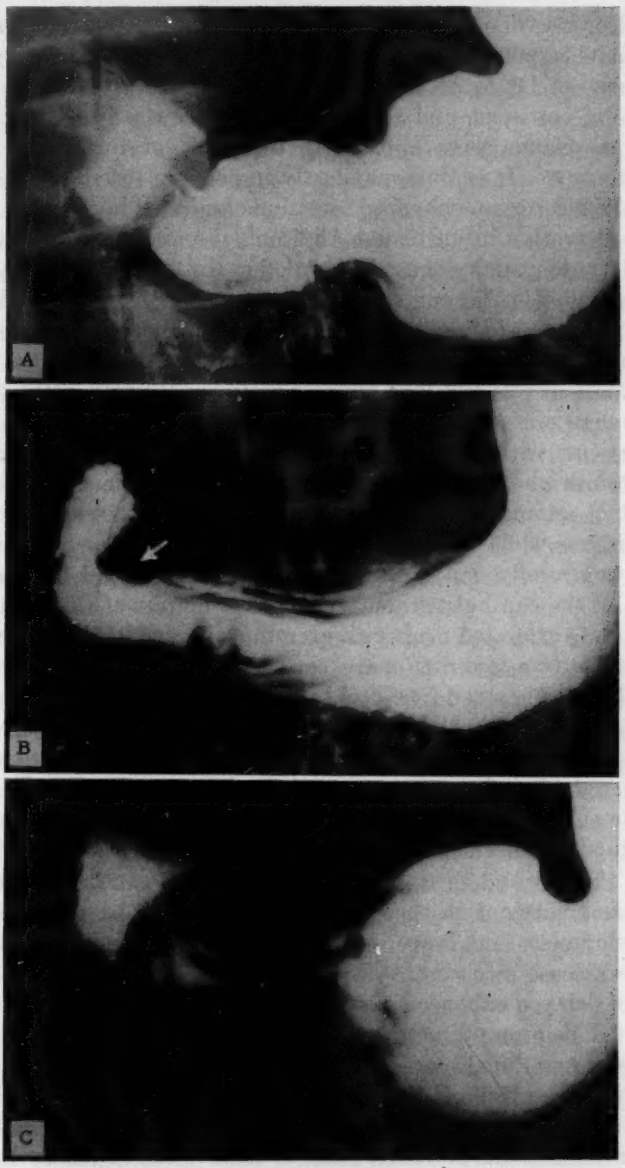

FIG. 1. Gastrointestinal x-ray films taken on (**A**) April 29, 1952, (**B**) January 6, 1955, and (**C**) February 15, 1955, demonstrating the appearance of the gastric ulcer on January 6, 1955, its healing, and the previously normal stomach.

gastric ulcer was demonstrated. She still showed no free acid on fasting, but a definite response to histamine. This was true whether she was receiving cortisone or DCA. At the time the ulcer developed, she was taking a fixed dose of cortisone, and the ACTH test performed later showed that her adrenal glands were apparently incapable of further secretion of adrenal hormone. It is thus manifestly impossible to relate the development of the ulcer to an enhanced secretion of adrenal hormone.

Peptic ulceration in untreated Addison's disease is excessively rare, as indicated by the paucity of cases recorded in the literature and by our own experience (10, 11). To our knowledge, this is the first reported example of peptic ulcer developing in a patient with Addison's disease who was receiving only maintenance cortisone therapy. In the only other recorded case, the patient was receiving massive cortisone therapy in the treatment of Addisonian crisis (12). However, Gray (personal communication) has seen 2 patients with gastric ulcers comparable to the present one, and 3 other patients who were known to have chronic duodenal ulcers prior to the onset of adrenal insufficiency in whom there was recurrence of epigastric distress while receiving a dosage of cortisone ranging between 25 and 37.5 mg. orally daily. All showed chronic duodenal deformity with irritation of the cap but without active ulcer craters at this time. Two of these patients exhibited uropepsin excretions in the duodenal ulcer range. All responded to a lowering of the cortisone dosage and to ulcer management and have remained free of ulcer symptoms since then.

Any concept relating the adrenal cortex and peptic ulcer must take into account the following facts: 1. The extreme rarity of peptic ulceration during untreated adrenal insufficiency and the finding of low gastric secretory activity in such cases. 2. The occasional occurrence or reactivation of gastric and duodenal ulcers in Addison's disease patients receiving maintenance cortisone therapy and the restoration to normal of gastric secretory activity by this treatment in the average patient with Addison's disease. 3. The development and exacerbation of peptic ulcer in patients without Addison's disease receiving ACTH or cortisone therapy and the demonstration of delayed enhanced gastric secretory activity in response to such therapy. 4. The prompt increase in gastric secretory activity in response to physical and emotional stress in many patients.

These facts correspond precisely with those which have related the response to nonspecific stress and the adrenal cortex. They have been considered in terms of the permissive role of the adrenal cortex by Ingle (5, 6) and ourselves (7, 8) and in terms of conditioning factors by Selye (9). Thus, it was found that certain metabolic responses to stress in rats did not occur in the absence of the adrenal cortex, but did when the animals were receiv-

ing a maintenance dose of adrenal extract or cortisone. If the animal with intact adrenals was given a dose of adrenal hormone and then exposed to a stressful stimulus, it responded with the typical metabolic reaction to stress, even when the stimulus was one which ordinarily was too feeble to elicit the response. In general, the metabolic response to stress occurred more rapidly than that to an excess of adrenal hormone. A similar sensitization to stress by cortisone was demonstrated when the carbohydrate and insulin tolerances of normal and ill subjects were compared before and after a single dose of cortisone (13). These data were interpreted to indicate that an excess of cortisone in some way sensitizes the organism to respond more rapidly and readily to stressful stimuli. Selye, basing his interpretation on similar types of experiments, refers to this phenomenon as the conditioning effect. He considers that the stress conditions the tissues to respond to adrenal hormone, rather than vice versa as proposed by ourselves.

Regardless of the precise mechanism by which the peptic ulceration itself is brought about, the facts in relation to the adrenal cortex may be interpreted reasonably in the same terms as the experimental data just described. Thus, adrenal hormone is presumably necessary for the initiation of the process of ulceration but is itself not the cause, in the sense of an excess of hormone having a specific necrosing effect by *itself*. In the presence of excess hormone, however, the gastric and duodenal mucosa might be thought of as being made more sensitive and responsive to those potentially noxious stimuli which, in the absence of excess hormone, would not be great enough to initiate ulceration. It is noteworthy in this regard that Gray found that gastric secretory activity responded very promptly to emotional and physical stress, whereas the response to ACTH generally was delayed from seven to fourteen days in individuals with presumably previously normal gastrointestinal function (3). Our own studies also demonstrated a temporal difference in the metabolic response to stress versus that to adrenal hormone, with the former occurring more rapidly. For the purpose of this discussion it is immaterial whether the primary stimulus is psychologic and mediated by neural and/or vascular changes, or whether it is local or mechanical.

This interpretation is compatible with a dualistic concept of the etiology of peptic ulcer, *i.e.*, with no change in adrenal hormone secretion (as in our patient and presumably in most individuals with normal adrenal function) certain stimuli are sufficient by themselves to initiate the process of peptic ulceration, whereas under other circumstances (hyperadrenalcorticism, either endogenous or exogenous) there may be an increase in the sensitivity of the gut to stimuli which would otherwise not be damaging. The normal student described by Hirschowitz *et al.*, in whom a previously undiagnosed

duodenal ulcer was activated ninety hours after administration of ACTH experimentally, may be a good example of the latter situation (14). Actually, however, there is no good evidence to indicate that hyperadrenalism per se is commonly an important factor in the production or activation of the average peptic ulcer (15). It is of interest that in a recent abstract Gray interprets the adrenal-gastric relationship in terms of the permissive concept (16).

This type of clinical interrelationship is not unique for peptic ulceration, as the same formulation might be made for other pathologic states—for example, hypertension. Patients with Addison's disease generally have hypotension. The blood pressure is restored to normal by cortisone therapy, but in an occasional patient hypertension develops, even during maintenance cortisone therapy. In certain individuals with presumably normal adrenal function hypertension develops when they receive ACTH or cortisone. Although hypertension occurs frequently in Cushing's syndrome, there is no good evidence to implicate the adrenal gland in the average case of essential or malignant hypertension. It may be presumed, then, that adrenocortical hormones are necessary for, but are not the cause of the elevation of blood pressure, and that in the presence of excess adrenal hormone the vascular system is more responsive to stimuli or agents which cause hypertension. This hypothesis is supported by the report that cortisone treatment sensitizes the vascular system to the hypertensive effect of norepinephrine (17).

This general type of relationship, which has been so well demonstrated in the laboratory, thus has broad application to the interpretation of many clinical situations.

SUMMARY

A 49-year-old Negro housewife with Addison's disease is described, in whom a gastric ulcer developed during treatment with 25 mg. of cortisone acetate daily. The significance of the occurrence of peptic ulcer in a patient with cortisone-treated adrenal insufficiency is discussed in the light of the concept of the permissive role of the adrenal cortex in disease. It is suggested that adrenal hormone is necessary for, but is not the cause of the development of peptic ulcer, and that in the presence of an excess of adrenal hormone the stomach and/or duodenum may be sensitized to respond by secretory hyperactivity and/or ulceration to stimuli which would ordinarily be innocuous.

FRANK L. ENGEL, M.D.

Department of Medicine,
Duke University,
Durham, N. C.

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Letters to the Editor

PREDNISONE AND PLASMA LIPIDS

To the Editor:

Elevation of serum lipids has been observed in man after prolonged administration of large doses of cortisone (1). Rabbits treated with cortisone and hydrocortisone showed lactescence of plasma, elevation of all lipid fractions, especially total lipid and neutral fat (2), and alteration of the lipoprotein pattern (3). This paper presents a comparative study of the effects of prednisone (1-dehydrocortisone), cortisone and hydrocortisone on plasma lipid partition (by chemical analysis) and on lipoprotein and protein patterns (by paper electrophoresis) in the rabbit. The methods were those previously used in this laboratory (2, 3).

Of 35 male rabbits, 12 were injected for thirty days with prednisone intramuscularly, 12 with cortisone acetate, and 11 with hydrocortisone acetate. The daily dose was 1.25 mg./Kg. for each hormone. Despite the comparatively small dose of the hormones, animals treated with cortisone and hydrocortisone exhibited frequent intercurrent infections and fatalities, necessitating replacement of about one third of these animals. Such complications were not encountered in the group of animals treated with prednisone. Thus, prednisone was, in equal dose, better tolerated by the rabbit than was cortisone or hydrocortisone.

There was a progressive increase of all plasma lipid fractions in the three groups of animals (Table 1). The increase in phospholipids and neutral fats occurred earlier and was more pronounced than that of cholesterol, resulting in moderate changes of the cholesterol:phospholipid ratio. The

Table 1. Effect of prednisone, cortisone and hydrocortisone on the plasma lipid partitions of the rabbit

Corticosteroid	0				10 Days				20 Days				30 Days			
	Chol. T/E*	P.L.	T.L.	N.F.	Chol. T/E	P.L.	T.L.	N.F.	Chol. T/E	P.L.	T.L.	N.F.	Chol. T/E	P.L.	T.L.	N.F.
Cortisone [12]†	55/41	112	306	139	59/37	154	655	442	69/29	212	1,090	809	75/45	181	822	566
Hydrocortisone [11]†	47/35	103	505	355	42/25	127	771	512	49/25	151	745	545	79/41	250	1,014	765
Prednisone [12]†	50/35	105	350	195	56/33	178	701	467	86/40	238	1,260	936	130/46	323	2,056	1,633

* T/E=total/esterified cholesterol. P.L.=phospholipid. T.L.=total lipid. N.F.=neutral fat. All values are expressed in mg./100 ml.

† The figures in brackets indicate the number of animals of each group.

increase in cholesterol affected to a greater extent the free than the esterified fraction. The esterified cholesterol fraction decreased from 70–75 per cent of the total cholesterol to 35–60 per cent after thirty days. The decrease of esterified cholesterol was most pronounced following prednisone administration. Similarly, the elevations of all lipid fractions were decidedly higher after twenty and thirty days of prednisone administration than after cortisone and hydrocortisone.

There was no change in plasma protein pattern in the three groups. However, there was a marked alteration in plasma lipoprotein patterns (Table 2), characterized by a decrease of the alpha and beta lipoproteins and a

TABLE 2. EFFECT OF PREDNISONE, CORTISONE AND HYDROCORTISONE ON PLASMA LIPOPROTEIN PATTERNS IN THE RABBIT

Corticosteroids	No. of animals	Days of treatment (avge.)	Lipoproteins (% of total stainable lipids)		
			Alpha	Beta	0
None	16	0	30.7	50.4	18.9
Cortisone	2	14	10.2	30.8	59.0
Hydrocortisone	2	29	11.8	33.9	54.3
Prednisone	5	30	5.8	34.5	62.7

concomitant increase of the 0-fraction (lipoprotein adhering to the point of origin). Again, these changes were most pronounced after prednisone administration.

The observations on the effect of prednisone on plasma lipids are of interest. A similar trend was noted previously after administration of cortisone and hydrocortisone (2). The elevation of the free plasma cholesterol level without a corresponding increase in the esterified fraction was seen in all groups of animals but was more pronounced during prednisone administration. These observations may indicate a metabolic block in the esterification of cholesterol as a part of the effect of these steroids on lipid metabolism. The marked increase in the level of plasma neutral fats may similarly be related to impaired esterification of fatty acids. In their studies on cortisone, Pierce and Bloom suggested a block of lipoproteins at the SF 40–80 level (4). The studies with labelled cholesterol and acetate in abnormal states of lipid metabolism in man also disclosed differences in the relation of plasma free and esterified cholesterol during the period of incorporation (5). Thus, the elevation of all plasma lipid fractions after prednisone, cortisone and hydrocortisone administration might be the result of increased synthesis, a metabolic block affecting esterification of fatty acids, or perhaps a combination of both mechanisms.

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CHUN-I WANG, M.D.
ELAINE T. BOSSAK, M.D.
DAVID ADLERSBERG, M.D.

The Departments of Medicine and Chemistry,
The Mount Sinai Hospital,
New York 29, N.Y.

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RECOVERY OF ALDOSTERONE, HYDROCORTISONE AND CORTISONE FOLLOWING DIALYSIS

TO THE EDITOR:

Using Zaffaroni's method (1) for the dialysis of steroids through cellulose casing, we have studied its application to the recovery from plasma of three $C_{21}O_5$ corticosteroids—hydrocortisone, cortisone and especially aldosterone. This work derives from our interest in the study of urinary and plasma aldosterone in normal subjects and in patients with arterial hypertension, with generalized edema and other metabolic diseases.

A few minor modifications were made in the original technique: 1) The cellulose sausage casing is tightly attached by a double knot at each end to a 1-cm. wide flexible stainless steel rack, to prevent its breakage during dialysis. 2) The chloroform phase is removed after twenty-four hours and

replaced by a fresh aliquot for a total dialysis time of seventy-two hours. 3) The outside phase of 40 per cent aqueous methanol is extracted after dialysis five times with 0.25 volume of chloroform. All the chloroformic extracts are then pooled. 4) The extract residue is partitioned between petroleum ether and 80 per cent aqueous methanol in order to remove pigments, fats and other materials which interfere with subsequent chromatography. No blue tetrazolium-reacting substances were found in the petroleum-ether phase, in the experiments involving dialysis from a 33 per cent aqueous methanol solution.

The purification and isolation of the corticosteroids studied is obtained by a double chromatographic procedure (2), first in Zaffaroni's propylene glycol/toluene system, and second in the Bush C cystem of toluene 90-ethyl acetate 10/50 per cent aqueous methanol. The ethanol-dissolved residue from the second chromatographic eluate is examined for its ultraviolet absorption curve in a Beckman model DU spectrophotometer. From the maximal optical density of the absorption peak between 238 and 242 mμ, the concentration of the corticosteroid studied is calculated against a similarly treated blank (from dialysis to chromatography) according to the following formula:

$$\text{Concentration (grams/liter)} = \frac{\text{Optical Density} \times \text{Molecular Weight}}{\text{Extinction Coefficient}}$$

Ten dialysis experiments were performed with cortisone and hydrocortisone in amounts varying between 100 and 2,200 micrograms, half from a plasma-water-methanol 1:1:1 solution, and half from a 33 per cent aqueous methanol solution. The results from these ten experiments showed a recovery of 94.2 to 102.4 per cent of the original amounts as determined by ultraviolet absoprtion. These observations were checked in two experiments by a micro blue-tetrazolium determination (3) and the same degree of recovery was found. This confirms the findings of Axelrod and Zaffaroni (4), and the more recent ones of Lombardo *et al.* (5) who used a slightly different outside phase (60 per cent) aqueous methanol and methylene chloride as the extracting solvent.

Three experiments involving the dialysis of 60.2 micrograms of aldosterone (two from plasma-water-methanol 1:1:1 solution, and one from 33 per cent aqueous methanol) showed recoveries of 99.8, 99.9 and 98 per cent, respectively, of the original amount. The excellence of the recovery of aldosterone after dialysis from plasma led us to use this method for the study of its plasma concentration in normal subjects and in patients. This will be the subject of a forthcoming publication.

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WOJCIECH NOWACZYNSKI, D.Sc.
JACQUES GENEST, M.D.

Département de recherches cliniques,
Hôtel-Dieu de Montréal,
3840, Rue St-Urbain,
Montréal 18, Canada.

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The Endocrine Society

THE 1956 ANNUAL MEETING

The Thirty-eighth Annual Meeting of The Endocrine Society will be held in the Palmer House, Chicago, June 7, 8 and 9, 1956.

THE 1956 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship was established in 1947. It is designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. The Fellowship is awarded on alternate years (1955, 1957, etc.) and the stipend, which will not exceed $5,000, may be

divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst, McKenna and Harrison Fellowship may be made by any member of the Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.